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VOL. 32 JUNE, 1947 No. 6

The Journal of Laboratory and Clinical Medicine

The Official Publication of the Central Society for Clinical Research

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Vol. 32, No. 6, June, 1947. The Journal of Laboratory and Clinical Medicine is published monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis (3), Mo. Subscription Price: United States, its Possessions, Pan-American Countries, $8.50 per year; Canada, $9.50; Foreign, $9.50. Subscriptions to medical students, interns and residents, half price. Entered as Second-Class Matter October 5, 1915, at the Post Office at St. Louis, Missouri, under Act of March 3, 1879. Printed in the U. S. A.

Hal Downey, Ph.D.

PROFESSOR OF HISTOLOGY AND HEMATOLOGY, 1917-1946
UNIVERSITY OF MINNESOTA, EMERITUS PROFESSOR, 1946—

IN HONOR OF DR. HAL DOWNEY

MY FRIEND, Professor Hal Downey, retired last June from active duty on the teaching faculty of the University of Minnesota. This event prompted former students and associates to dedicate this issue of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE to him as a mark of esteem and gratitude. They asked me to join them in this token of respect.

I am one of the few survivors of the old school of nonclinical hematology and had the privilege of working with Dr. Downey when he visited Europe in 1911, with the intention of extending his earlier studies on the blood-forming organs of the lower vertebrates (Polyodon in particular) to mammals and man. He went first to Pappenheim in Berlin. But Pappenheim's more philosophical mind and mode of viewing morphological problems were not entirely to Downey's taste. So he left Berlin and went to Strassburg to work in my laboratory. The equipment there was very poor and no technical assistance was available. We had only the simplest microscopical techniques with which to tackle the problem of the origin of the lymphocytes—which was our goal.

When Downey left me, I gave up hematology and never returned to it. Downey, however, remained faithful to the subject. Acknowledging that the clue to the lymphocyte problem, the relation between lymphoblasts and myeloblasts, is only approachable through experiment—that is, changing the normal environment of the cells—he decided to study the blood cells under their pathological conditions.

I do not feel qualified to praise Dr. Downey's achievement in the field of clinical hematology and must leave this to more competent persons. But I am very proud of having served Dr. Downey as a guide at the beginning of a career in which he has become one of the foremost hematologists of our age.

—*Franz Weidenreich.*

AN EVALUATION OF ALTERATIONS PRODUCED IN LYMPHOID TISSUE BY PITUITARY-ADRENAL CORTICAL SECRETION

THOMAS F. DOUGHERTY AND ABRAHAM WHITE
NEW HAVEN, CONN.

THE involvement of lymphoid tissue and of lymphocytes in numerous normal and pathologic processes is well recognized by pathologists and experimental investigators. However, the relation of the experimental and pathologic findings to the actual function of the lymphocyte has remained obscure. The normal physiologic control and function of this ubiquitous tissue has been largely speculative. Perla and Marmorston,[1] in discussing lymphoid tissue, ask the following questions: "Why does the mammal possess so large and diffuse an organ (lymphoid tissue)? What is the purpose of this mass of lymphoid tissue? Why are such large numbers of lymphocytes poured into the circulation daily, without causing a fluctuation in the lymphocytic count of the blood? Where do the lymphocytes go? What is the function of the lymphocyte?"

Drinker and Yoffey[2] have stated that the only definitely known function of lymphoid tissue is the production of lymphocytes. Actually, the origin of the lymphocyte is still a matter of controversy among some histologists. The multipotentiality of the lymphocyte has been demonstrated.[3] Lymphocytes may be sources of immature cells, which transform to specialized cellular forms when the necessity arises, or a reservoir of potential macrophages. However, these roles are potentialities of the lymphocyte; the normal functions of the cell morphologically identified as a lymphocyte have not been elucidated.

The constancy in the size of the lymphoid tissue and in the numbers of circulating lymphocytes suggest the possibility that the rate of growth of lymphocytes and their entry into the circulation may be under endocrine control. It is not unlikely that these processes may be intimately related also to factors concerned with the peripheral disposition of lymphocytes. Addison noted an increase in lymphoid tissue in his original description of the disease which bears his name (quoted by Grollman[5]). Conversely, numerous investigators have found that the secretions of the adrenal cortex have a regulatory effect on the size of the thymus.[6-12] This paper includes some of the data obtained in studies initiated to investigate the hypothesis that pituitary adrenotrophic hormone (adrenotrophin), by its action on the adrenal cortex, influences the size, structure, and function of lymphoid tissue.

EXPERIMENTAL OBSERVATIONS

Pituitary-Adrenal Cortical Control of Lymphoid Tissue Weight.—The daily subcutaneous injection of 1 mg. of adrenotrophin[13] in 0.5 c.c. aqueous solution

From the Departments of Anatomy and Physiological Chemistry, Yale University.
The data reported in this paper have been obtained in studies aided by grants from the Josiah Macy, Jr., Foundation and the Fluid Research Fund of Yale University School of Medicine.

into normal CBA mice, 60 to 80 days old, resulted in a significant decrease in the weight of lymphoid tissue. This decrease was observed as early as fifteen hours following injection and persisted throughout the fifteen-day period during which the mice were treated[14] (Figs. 1 and 2). The thymus remained smaller than other lymphoid structures during the period of study. Although the spleen actually increased in weight during the fifteen days of hormone administration, this increase was due to the accumulation of large numbers of degenerating lymphocytes and to an extensive edema. The Malpighian corpuscles were markedly decreased in size. The histologic changes in these tissues will be discussed later. Adrenotrophin has now been reported to produce similar weight changes in lymphoid organs of the rat.[15]

Adrenalectomized mice maintained for eight days by daily, subcutaneous injections of 0.025 mg. desoxycorticosterone acetate in oil (Schering), showed marked hyperplasia of all the lymphoid organs studied (Figs. 1 and 2). The alterations in lymphoid tissue following adrenalectomy were not influenced by desoxycorticosterone acetate since the maintenance dose of this steroid produced no effect on the weight or histology of lymphoid tissue in normal or adrenalectomized mice.[16, 17]

Pituitary Control of Adrenal Size and Cholesterol Content.—Repeated daily injections of adrenotrophic hormone resulted in the expected increase in weight of the adrenals (Fig. 2). The cholesterol content of the adrenals of hormone-treated mice varied during the fifteen-day period, depending on the particular day the glands were obtained. Whereas a single injection of hormone produces an acute decrease in adrenal cholesterol within a few hours after administration[18] (Fig. 3), repeated daily injections increase the adrenal cholesterol to values above normal.[19]

Pituitary-Adrenal Cortical Control of the Numbers of Circulating Lymphocytes.—In the fifteen-day experiments previously described animals were sacrificed in groups at intervals of three days. All of these mice were killed approximately twenty-four hours after the last injection of adrenotrophic hormone. In addition to weighing the tissues, red and white blood cell counts and differential counts were made. Total hemoglobin and whole blood specific gravity were also determined. The data are summarized in Table I. The number of circulating lymphocytes in the adrenotrophic hormone-treated mice was significantly lower throughout the fifteen-day period.[20]

Similar studies have been conducted on adrenalectomized mice maintained on daily injections of 0.025 mg. desoxycorticosterone acetate over an eight-day period and on adrenalectomized rats given 0.7 per cent sodium chloride and 0.3 per cent sodium bicarbonate as drinking fluid. These data are presented in Tables I and II. In adrenalectomized mice and rats the number of lymphocytes increased significantly.[20] In the rats there was a significant lymphocytosis at six days following adrenalectomy, whereas mice had a lymphocytosis twenty-four hours after operation. Furthermore, the specific gravity of the whole blood of adrenalectomized mice maintained with desoxycorticosterone did not indicate a hemoconcentration sufficient to account for the increased number of lymphocytes (Table I). The number of circulating lymphocytes in adrenalectomized

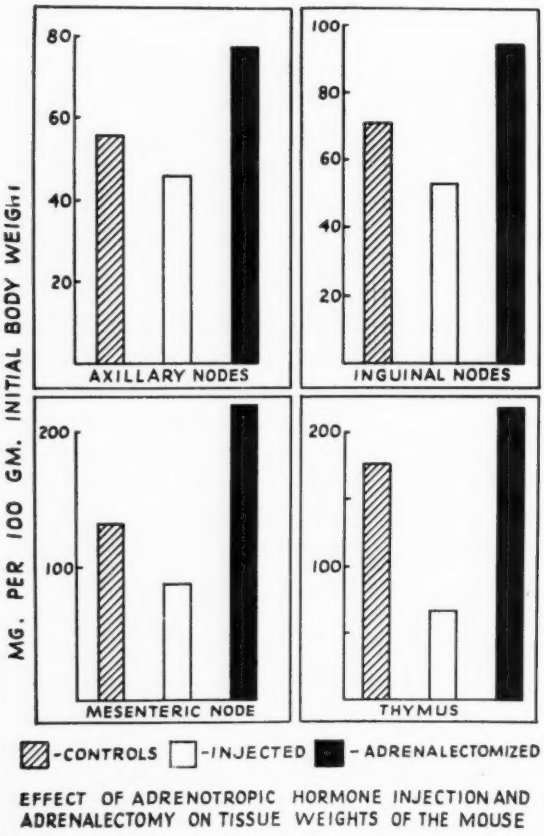


Fig. 1.—The columns represent weights of paired lymph nodes, adrenals, and testes of mice, 60 to 80 days old, of the CBA strain (Strong). The control group represents thirty mice, and there were twenty-five mice in each of the experimental series. The adrenotrophic hormone-injected mice were treated daily with 1.0 mg. of hormone for fifteen days; the adrenalectomized mice were sacrificed one week after adrenalectomy.

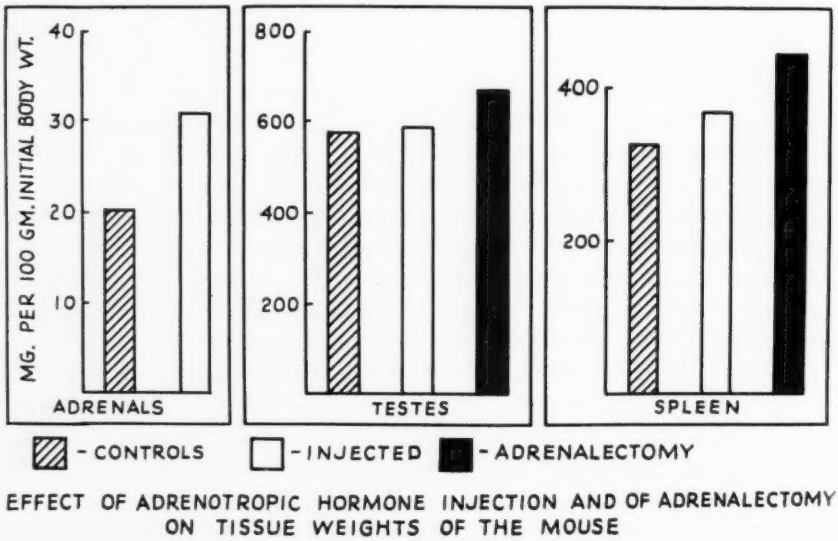


Fig. 2.—Same as Fig. 1.

TABLE I. EFFECT OF CONTINUED INJECTION OF ADRENOTROPHIC HORMONE AND OF ADRENALECTOMY ON CERTAIN BLOOD CONSTITUENTS OF THE MOUSE*

	CONTROL—UNINJECTED ANIMALS	ADRENOTROPHIC HORMONE-INJECTED ANIMALS 1 MG. DAILY 15 DAYS	P VALUES†	ANDRENALECTOMIZED ANIMALS	P VALUES†	ADRENALECTOMIZED ANIMALS + DCA	P VALUES†
Number of animals	99	30		14		32	
Hemoglobin (Gm. %)	15.3 ± 0.62‡	16.4 ± 0.25	< 0.01	17.5 ± 0.27	< 0.01	15.9 ± 0.18	0.06
R.B.C. (mil./c.mm.)	9.05 ± 0.50‡	10.15 ± 0.19	< 0.01	9.73 ± 0.47	< 0.01	9.54 ± 0.25	0.10
W.B.C.	12,814 ± 435	11,749 ± 737	0.25	17,025 ± 1,682	< 0.01	15,761 ± 819	< 0.01
Lymphocytes	9,584 ± 371	6,932 ± 510	< 0.01	14,549 ± 1,514	< 0.01	11,674 ± 684	< 0.01
Polymorphonuclear leucocytes	3,257 ± 158	4,694 ± 358	< 0.01	2,476 ± 287	0.07	3,087 ± 397	0.6
Specific gravity whole blood	1.060 ± 0.0006§	1.063 ± 0.0058‖	0.05	1.068 ± 0.0013	< 0.01	1.064 ± 0.0002	< 0.01

*Values in this tables are means and standard errors.
†P values as compared to controls.
‡ Thirty-nine animals.
§Nineteen animals.
‖Ten animals.

TABLE II. EFFECT OF ADRENALECTOMY ON CERTAIN BLOOD CONSTITUENTS OF THE RAT*

	CONTROL—UNINJECTED ANIMALS	TWO DAYS AFTER ADRENALECTOMY	P VALUES†	FOUR DAYS AFTER ADRENALECTOMY	P VALUES†	SIX DAYS AFTER ADRENALECTOMY	P VALUES†
Number of animals	42	13		9		9	
Hemoglobin (Gm. %)	14.8 ± 0.14‡	14.0 ± 0.35	0.01	13.4 ± 0.27	< 0.01	12.8 ± 0.38	< 0.01
R.B.C. (mil./c.mm.)	8.88 ± 0.23‡	9.38 ± 0.31	0.25	8.02 ± 0.18	0.10	7.93 ± 7.73	0.05
W.B.C.	17,826 ± 809	17,194 ± 1,748	0.70	19,011 ± 2,016	0.50	26,082 ± 1,825	< 0.01
Lymphocytes	14,700 ± 724	13,946 ± 1,182	0.60	15,739 ± 1,597	0.50	20,011 ± 1,201	< 0.01
Polymorphonuclear leucocytes	2,932 ± 734	4,163 ± 274	0.05	3,272 ± 839	0.50	6,072 ± 921	< 0.01
Specific gravity whole blood	1.059 ± .00004§	1.058 ± .001	0.80	1.057 ± .0009	0.90	0.056 ± .0002	0.90

*Values in this table are means and standard errors.
†P values as compared to controls.
‡Twenty-seven animals.
§Eight animals.

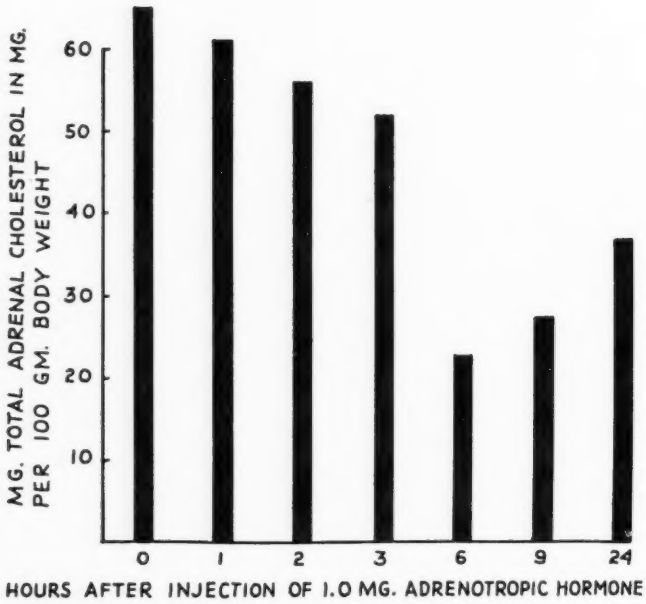


Fig. 3.

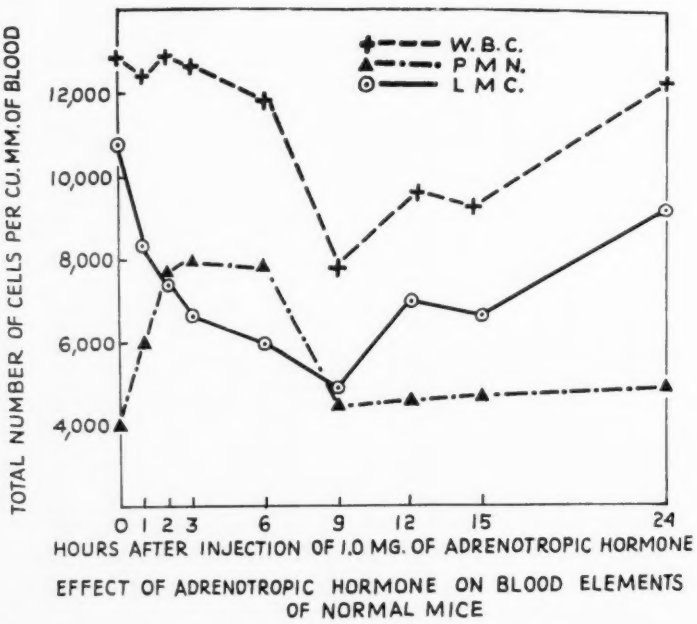
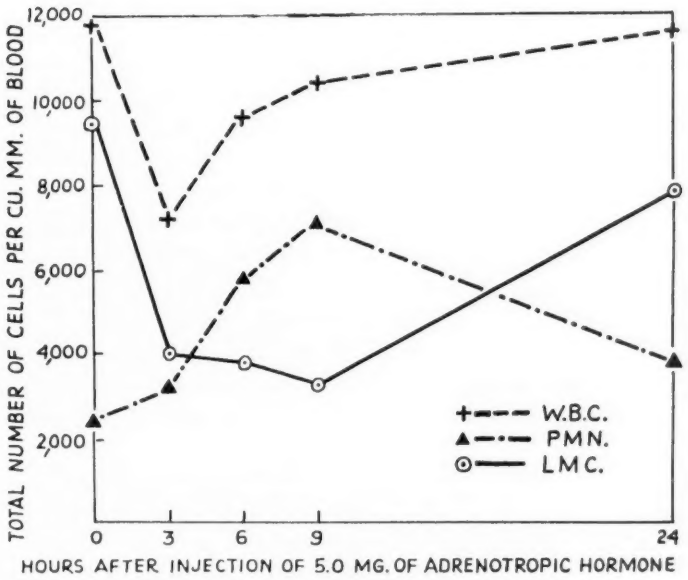
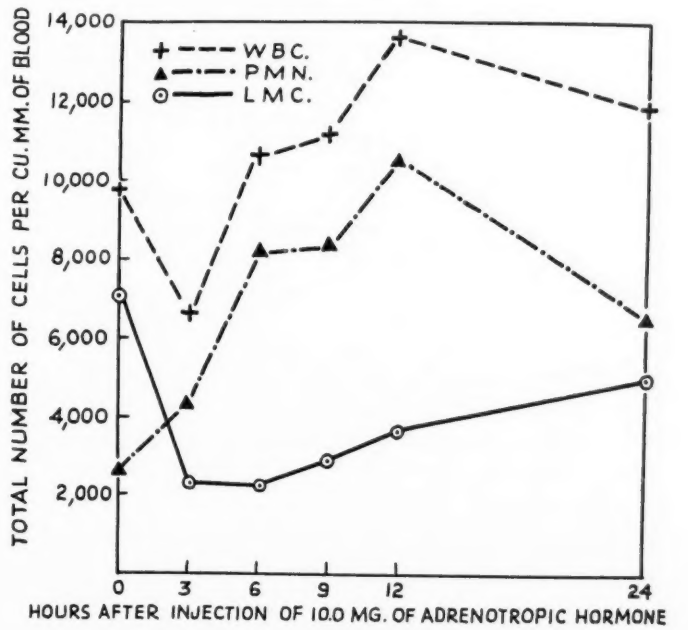

TOTAL NUMBER OF CELLS PER CU. MM. OF BLOOD
W. B. C.
P M N.
L M C.
12,000
10,000
8,000
6,000
4,000
0 1 2 3 6 9 12 15 24
HOURS AFTER INJECTION OF 1.0 MG. OF ADRENOTROPIC HORMONE
EFFECT OF ADRENOTROPIC HORMONE ON BLOOD ELEMENTS OF NORMAL MICE

Fig. 4.—Alterations produced in total leucocyte, lymphocyte, and polymorphonuclear cells in blood of normal mice receiving at zero time a single subcutaneous injection of 1.0 mg. of pituitary adrenotrophic hormone. The averages at each successive time interval are for groups of 99, 3, 3, 7, 7, 7, 6, 7, and 7 animals.



EFFECT OF ADRENOTROPIC HORMONE ON BLOOD ELEMENTS OF NORMAL RATS

Fig. 5.—Alterations produced in total leucocyte, lymphocyte, and polymorphonuclear cells in blood counts of normal rats receiving at zero time 5.0 mg. of adrenotrophic hormone subcutaneously. The averages at each successive time interval are for groups of 42, 12, 12, 8, and 12 animals.

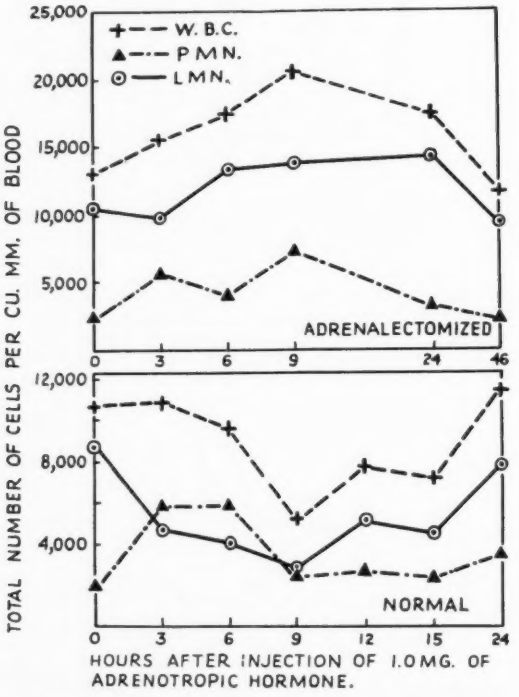


EFFECT OF ADRENOTROPIC HORMONE ON BLOOD ELEMENTS OF NORMAL RABBITS

Fig. 6.—Alterations produced in total leucocyte, lymphocyte, and polymorphonuclear cells in blood counts of normal rabbits receiving at zero time 10.0 mg. of adrenotrophic hormone subcutaneously. The averages at each successive time interval are for groups of 18, 4, 4, 4, 2, and 3 animals.

rats provides further evidence that the increase in lymphocyte counts in the operated mice is not due to hemoconcentration, since the adrenalectomized rats had an approximately normal blood volume and yet showed a lymphocytosis (Table II).

Significance of Lymphocyte Counts as an Index of Adrenal Cortical Activity.—The slight, but significant, lymphopenia in mice twenty-four hours after the previous injection of adrenotrophin suggested that the numbers of circulating lymphocytes might be an index of the level of adrenal cortical activity. On the basis of the adrenal cholesterol analyses shown in Fig. 3, maximum adrenal cortical activity occurred at six hours following a single injection of adrenotrophic hormone. Therefore, the blood of mice, rats, rabbits, and dogs was studied at intervals shortly following subcutaneous injection of this hormone.



EFFECT OF ADRENOTROPIC HORMONE ON THE WHITE BLOOD CELL COUNTS OF NORMAL AND OF ADRENALECTOMIZED MICE

Fig. 7.—Alterations produced in the leucocytes of adrenalectomized mice receiving at zero time 1.0 mg. of adrenotrophic hormone subcutaneously. The lower chart for normal animals is reproduced for comparison. The averages represent sixty control animals at zero time and five at each successive interval.

A single subcutaneous injection of 1.0 mg. of adrenotrophin in mice produced a precipitous decline in the numbers of circulating lymphocytes which was evident within one hour and reached its lowest point within nine hours (Fig. 4). Following this time, the numbers of blood lymphocytes rapidly increased and at twenty-four hours after hormone administration had returned

almost to normal. The lymphopenic response also was observed in rats injected with 5 mg. of adrenotrophic hormone (Fig. 5) and in dogs given 10 mg. of the hormone. In rabbits receiving 10 mg. of adrenotrophin the numbers of lymphocytes again decreased very rapidly, reaching their lowest value within three hours, and did not return to normal levels as rapidly as in the other species (Fig. 6).

The lymphopenic response produced by adrenotrophic hormone is dependent upon the mediation of the adrenal cortex. The hormone has no effect on blood lymphocytes in adrenalectomized animals (Fig. 7). Further, the specific hormonal action of adrenotrophin is seen from the fact that two other pure proteins, prolactin[21] and serum gamma globulin (human), had no effect on blood lymphocyte levels.

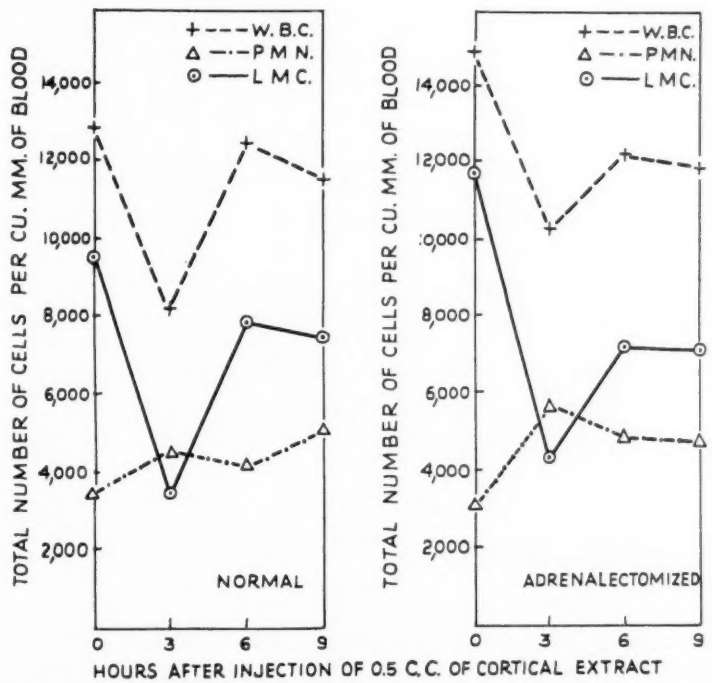


Fig. 8.—Effect of subcutaneous injection of 0.5 c.c. aqueous adrenal cortical extract on the leucocyte counts of normal and adrenalectomized mice. There are eight animals at each interval in the unoperated series and six animals at each interval in the adrenalectomized group.

Lymphopenic Response Produced by Various Adrenal Cortical Preparations.—The role of the adrenal cortex as a mediator in the lymphopenic effect of adrenotrophic hormone led to a study of various adrenal cortical hormone preparations. It was of considerable interest to determine which adrenal cortical steroids might be primarily responsible for the lymphopenia. Single injections of aqueous adrenal cortical extract (Wilson) or of adrenal cortical steroids in oil (Upjohn) produced lymphopenic responses in normal and adrenalectomized mice and rats[18] (Fig. 8 and Table III). These same adrenal cortical preparations were also effective in rabbits and in man. Corticosterone

and compound E (Kendall) produced lymphopenia in adrenalectomized mice (Table III). On the other hand, desoxycorticosterone acetate, in the doses used, did not produce lymphopenia in either normal or adrenalectomized mice (Table III). This lymphopenic effect of adrenotropin and of certain adrenal cortical preparations has now been confirmed by other investigators in rats,[22, 23] dogs,[24] and human beings.[25, 26]

TABLE III. AVERAGE ALTERATIONS IN BLOOD CONSTITUTENTS FOLLOWING SINGLE, SUBCUTANEOUS INJECTIONS OF ADRENAL CORTICAL STEROIDS IN NORMAL AND IN ADRENALECTOMIZED MICE

PREPARATION AND ANIMAL USED	NUMBER OF ANIMALS	DOSE (MG.)	TIME AFTER INJECTION (HOURS)	W.B.C.	LYMPHOCYTES	POLYMORPHONUCLEAR LEUCOCYTES
Desoxycorticosterone acetate in sesame oil injected into normal mice	99	0.1	0	12,814	9,854	3,257
	5		3	13,680	7,929	5,751
	3		6	16,600	9,339	7,260
	3		9	17,667	9,266	8,400
Desoxycorticosterone acetate in sesame oil injected into normal mice	3	0.25	0	12,814	9,854	3,257
	3		3	26,700	13,269	13,431
	3		6	28,500	13,190	15,310
	3		9	15,050	8,995	6,065
Desoxycorticosterone acetate in sesame oil injected into adrenalectomized mice	60	0.0	0	14,903	11,717	3,189
	3		3	13,833	9,133	4,693
	3		6	10,700	8,640	2,060
	3		9	18,375	14,591	3,784
Desoxycorticosterone acetate in sesame oil injected into adrenalectomized mice	60	2.5	0	14,903	11,717	3,189
	3		3	16,033	10,357	5,676
	3		6	16,100	10,280	5,820
	3		9	13,517	10,277	3,263
Adrenal cortical steroids in oil injected into normal mice	99	0.1	0	12,814	9,854	3,257
	7		3	18,093	3,158	4,935
	3		6	18,933	10,088	8,845
	3		9	16,633	10,680	5,953
Adrenal cortical steroids in oil injected into adrenalectomized mice	60	0.1	0	14,903	11,717	3,189
	9		3	11,617	7,430	4,292
	3		6	13,617	12,157	1,460
	3		9	20,000	17,323	2,677
Compound F (Wintersteiner) injected into aqueous solution into adrenalectomized mice	60	0.25	0	14,903	11,717	3,189
	6		3	24,567	14,009	10,560
	6		6	14,767	6,733	8,034
	6		9	11,040	7,282	3,756
	3		24	14,488	10,030	4,387
Corticosterone injected in aqueous solution into adrenalectomized mice	60	0.25	0	14,903	11,717	3,189
	6		3	20,575	11,037	9,638
	6		6	15,400	8,050	7,356
	6		9	16,000	11,172	4,827
	3		24	16,767	10,560	6,207

Pituitary-Adrenal Cortical Mediation of the Lymphopenia Induced by Stresses.—A wide variety of physical, chemical, and physiologic stresses have long been known to produce a lymphopenic effect. The literature pertaining to this subject will be discussed subsequently. It was considered possible that some of these stresses might cause a lymphopenia as a result of pituitary-adrenal cortical stimulation. Data have been obtained confirming this hypothesis. The following types of stresses have been studied: a single injection of arsenite, benzene, histamine, pitressin, or typhoid vaccine; exposure to cold; and inanition. When exposed to any of these stresses, normal mice or rats re-

sponded with a lymphopenia, whereas adrenalectomized mice or rats showed no decrease in lymphocyte counts. Moreover, neither pitressin nor benzene induced lymphopenia in hypophysectomized rats. Some of these findings are shown in Figs. 9 and 10.

Alterations Seen in Numbers of Polymorphonuclear Leucocytes.—The term polymorphonuclear leucocyte is used here as a matter of convenience. The segmented forms should probably be referred to as heterophils in the mouse and the rat and as pseudoeosinophils in the rabbit. Other polymorphonuclear forms such as the basophil and the eosinophil are not grouped separately since they were few in number.

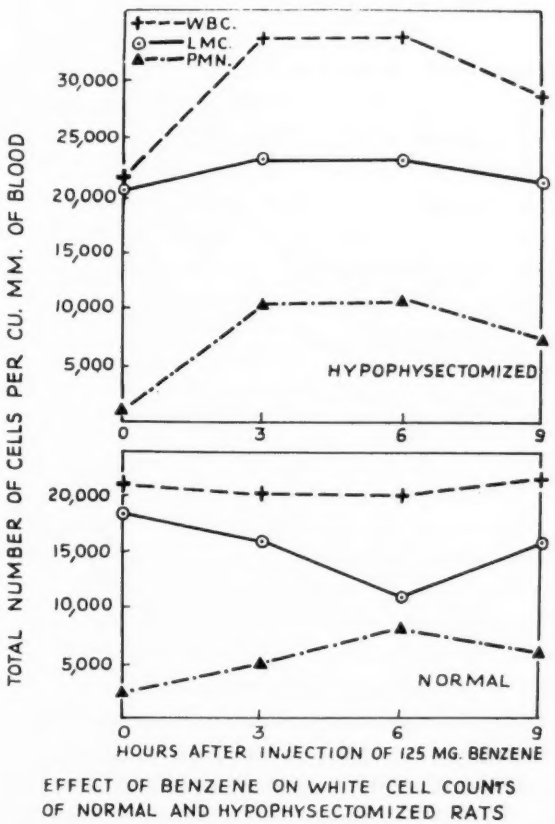
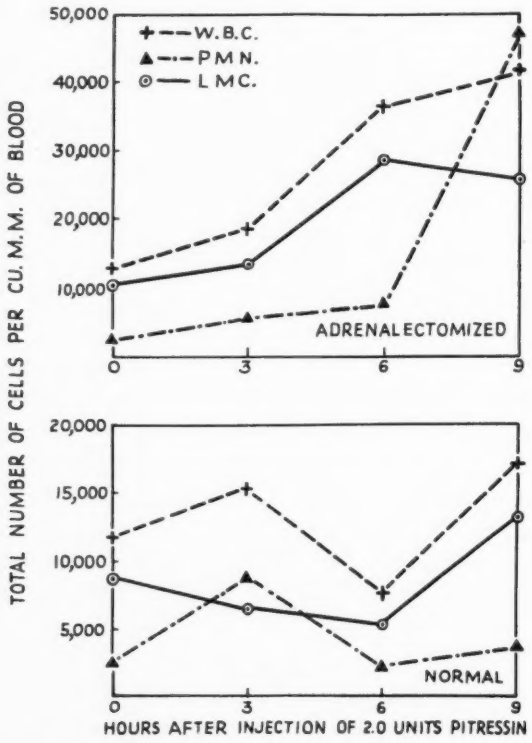


Fig. 9.—Effect of subcutaneous injection of 125 mg. benzene on the leucocyte counts of normal and hypophysectomized rats. Averages represent four animals at each interval in the hypophysectomized series and six animals in the normal series.

A slight but definite increase in the number of polymorphonuclear leucocytes was found in the mice given repeated injections of adrenotrophic hormone. It is questionable whether this increase was specifically related to the injection of the hormone, since a single injection of many agents, including adrenotrophin, produces an increase in circulating polymorphonuclear leucocytes when administered to either normal, adrenalectomized, or hypophysectomized animals (Figs. 7 to 10). The number of polymorphonuclear cells was lower, but not signifi-

cantly so, in the adrenalectomized mice. These cells were, however, increased in number in the adrenalectomized rats. In view of the fact that the polymorphonuclears did not increase in the blood of adrenalectomized mice, in spite of the hemoconcentration, it would seem that there was an actual suppression in the production or delivery of polymorphonuclears. A possible role of the adrenal cortex in the regulation of blood polymorphonuclear cells is suggested also by the work of Corey and Britton[27] who found a markedly decreased number of these cells in adrenalectomized cats. Pertinent discussion of this point has been presented previously.[18]



EFFECT OF PITRESSIN ON THE WHITE BLOOD CELL COUNTS OF NORMAL AND OF ADRENALECTOMIZED MICE

Fig. 10.—Alterations in numbers of leucocytes of adrenalectomized and normal mice following the subcutaneous injection of 2.0 units of pitressin. The control values are averages of ninety-nine mice in the unoperated group and forty-two in the adrenalectomized series. The values at each successive time interval were obtained on groups of six animals.

Pituitary-Adrenal Cortical Control of the Numbers of Circulating Erythrocytes.—Repeated injections of adrenotrophic hormone into normal mice resulted in a slight, but significant, polycythemia.[20] On the other hand, adrenalectomized mice, showing some degree of hemoconcentration despite daily injections of desoxycorticosterone acetate, had normal numbers of erythrocytes. Further, the significant decrease of red cells in adrenalectomized rats maintained with a normal whole blood specific gravity is additional evidence that pituitary-

adrenal cortical secretion exerts an influence over the numbers of circulating erythrocytes. The values for hemoglobin concentrations support these conclusions (Tables I and II).

In mice and rats given single injections of adrenotrophin, an initial increase in both hemoglobin concentration and red cell count was observed three hours after injection.[18] Thereafter, these constituents of the blood gradually declined, and twenty-four hours after hormone injection the red cells and hemoglobin concentrations were distinctly lower than normal. The specific gravity of the whole blood was not significantly altered during the twenty-four-hour period of study, although at the twenty-fourth hour there was evidence of a slight hemodilution.

Adrenal cortical preparations which produced a lymphopenia increased red cell counts and hemoglobin concentrations in a manner similar to that described for adrenotrophic hormone.

Histologic Changes in Lymphoid Tissue Produced by Adrenal Cortical Secretion.—The marked lymphopenia observed shortly following the injection of adrenotrophic or adrenal cortical hormones, or following a variety of stresses known to augment pituitary-adrenal cortical secretion, raised the problem of the mechanism inducing this lymphopenia. Several possibilities suggested themselves: failure of delivery of lymphocytes to the circulation, failure of lymphocyte production, or increased rate of peripheral removal of lymphocytes. The basis for the lymphopenia was sought in a detailed study of the lymphoid tissues following hormone administration.

In all the lymphoid tissues studied, that is, thymus, lymph nodes, spleen, and Peyer's patches, a single injection of adrenotrophic or of adrenal cortical hormones resulted in early dissolution of medium-sized and small lymphocytes within the lymphoid tissues. The dissolution of lymphocytes was accompanied by a marked edema. The term dissolution of lymphocytes has been used in the sense of a disintegration characterized by karyorrhexis pycnosis, or by a shedding of the cytoplasm of the cell.[16, 17] Downey and Weidenreich[28] have shown that under normal conditions lymphocytes may bud off portions of their cytoplasm. All of these alterations may be seen in the lymphoid tissues at the time of dissolution of lymphocytes. Certainly, the pycnosis and the disintegration of the nuclei of the lymphocytes result in death of these cells. However, many of the cells have markedly shrunken nuclei which have lost their normal chromatin distribution and have assumed various bizarre shapes. Although the cytoplasm of many of these cells has been lost, it is not to be construed that these cells are necessarily nonliving. It is possible that cells of this type might regenerate cytoplasm and again become normal lymphocytes.

In addition to the alterations in individual lymphocytes, there was an increased development of immature plasma cells similar to the cells described by Rohr[29] or the "acute splenic tumor cell" of Rich, Lewis, and Wintrobe.[30] Investigations in our laboratory have demonstrated[31] that the increase in these cells occurs within the first few hours following hormone injection in rabbits. It has also been observed that not only is there an increased number of so-called

immature plasma cells, but that many smaller lymphocytes may develop basophilic cytoplasm, thus giving to these cells the superficial appearance of plasma cells.

The dissolution of lymphocytes is maximal between one and six hours after hormone injection in both mice and rabbits. This is then followed by a removal of cellular debris by macrophages. These macrophages are numerous in all of the lymphoid organs studied and contained large amounts of nuclear debris. Concomitant with the dissolution of lymphocytes, early alterations in fixed reticulum cells are evident. These changes consist in an increase in the amount and structure of cytoplasm of the histiocytes. Although these cells were derived from fixed reticulum cells, they are not highly active in removal of the vast amount of nuclear debris.

Within six to nine hours after administration of either adrenotrophic or adrenal cortical hormones, most of the nuclear fragments scattered about in the lymphoid tissues had been phagocytized, and the tissues in most of the animals at this time showed little evidence of further lymphocyte dissolution. During this period and subsequently the edema subsided and there was evident a beginning return to normal lymphoid structure. The period of recovery was characterized by differentiation of lymphocytes from reticulum cells and resumption of mitosis in lymphocytes. Within twenty-four hours after hormone injection, the lymphoid structures, with the exception of the thymus, contained normal numbers of lymphocytes. These described changes in lymphoid structures as a result of increased concentration of circulating adrenal cortical hormone have been confirmed by Yoffey.[23]

The high degree of specificity of the acute action of adrenal cortical steroids on the lymphocytes of animals is seen from the fact that bone marrow elements other than lymphocytes showed no evidence of degeneration. *Thus, lymphocytes represent an important site of action of certain adrenal cortical steroids.*

Examination of other tissues such as the lung showed that the lymphopenia was not due to an accumulation of the lymphocytes in any of the large capillary beds. None of the other tissues examined were edematous.

Adrenotrophic hormone injected into adrenalectomized mice had no effect on lymphoid tissue. Adrenal cortical extracts (aqueous or oil), corticosterone, or compound E when injected into adrenalectomized mice produced histologic alterations indistinguishable from those seen after administration of these preparations to normal animals. On the other hand, desoxycorticosterone acetate injection into normal or adrenalectomized mice had no effect on lymphoid tissue structure in the doses employed (0.025 to 2.5 mg.). Recently, it has been reported[32] that large doses of desoxycorticosterone acetate produced an atrophy of the thymus, but not of other lymphoid structures, in adrenalectomized rats.

A more detailed description of the histologic alterations resulting from increased concentrations of circulating adrenal cortical steroids has been published.[17] Any one of the nonspecific stimuli studied, and mentioned previously in connection with alterations in numbers of blood lymphocytes, induced alterations in lymphoid tissue in normal mice similar to those described for adreno-

trophic hormone or for the effective adrenal cortical preparations. However, these same stimuli, in the degree employed, had no effect on the lymphoid tissue of adrenalectomized mice.

Adrenal Cortical Control of Release of Blood Globulin From Lymphocytes.—The dissolution of lymphocytes observed following injection of adrenotrophic or adrenal cortical hormones suggested that a portion of the released cytoplasmic nitrogen could enter the metabolic pool and participate in physiologic processes involving nitrogen. A portion of the nitrogen released by lymphocyte dissolution was sought in the serum proteins. Since lymphoid tissue has been suggested as a site of antibody production (for a review of the literature see Perla and Marmorston[1]), examination of the globulin fraction of the serum was of particular interest.

The daily injection of 1 mg. of adrenotrophic hormone into normal mice for fifteen days produced a statistically significant increase in the total serum proteins. On the other hand, adrenalectomy in the mouse was followed by a significant decrease in the level of total serum proteins. This decrease was even more significant when considered in the light of the slight degree of hemoconcentration which occurred in the adrenalectomized mice despite the daily administration of small amounts of desoxycorticosterone acetate. A single injection of 3.0 mg. of adrenotrophin into rats resulted in a significant increase in the total serum proteins at three-hour and six-hour periods after hormone injection at which the sera were studied. At twenty-four hours after hormone, the level of serum proteins had returned to normal values.[16, 33, 34]

The injection of either adrenotrophic hormone or adrenal cortical steroids into normal rabbits produced a significant increase in the total globulin content of the serum during the three to twenty-four hour period that the animals were studied. Electrophoretic patterns revealed that the increase in globulin was a result of rises in both the beta and gamma globulin components of the rabbits' sera.[33, 34] The albumin and the alpha globulin fractions were unaltered. These data assume added significance in the light of the demonstration that washed lymphocytes from the lymphoid tissue of normal rabbits contain a protein which has an electrophoretic mobility identical with that of the normal gamma globulin of rabbits' blood.[33, 34] Kass,[35] working with human lymphoid tissue, has confirmed the presence in lymphocytes of a protein immunologically identical with normal serum gamma globulin.

A second component of lymphocyte extracts is probably identical with the beta globulin of rabbits' blood. It is, therefore, established that normal lymphocytes contain at least one, and possibly two, proteins which are indistinguishable from certain of the normal globulins of the blood, and that these proteins are released from lymphocytes to the circulation under circumstances of augmented pituitary-adrenal cortical secretion.[34-36]

Adrenal Cortical Control of the Release of Antibodies From Lymphocytes.—The demonstrated release of globulin from lymphocytes by adrenotrophic and adrenal cortical hormones suggested that these hormones might stimulate an increased production of antibodies in immunized animals. The daily administration of adrenal cortical extract to mice, rats, and rabbits being immunized

with a variety of antigens produced a higher level of antibody in the sera of those animals injected with hormone as compared to the sera of animals receiving antigen alone.[37, 38]

Because antibodies have been demonstrated in lymphocytes,[39, 40] augmented dissolution of these cells by adrenal cortical steroids would be expected to increase titer. In hyperimmunized rabbits a single injection of adrenal cortical extract (aqueous or oil), or of adrenotrophic hormone, resulted within six hours in a marked rise in antibodies in the blood.[37, 38] The maximum effect was observed at nine hours, following which time the titers gradually returned approximately to their previous levels. The data establish that higher titers of antibody appear in the immunized animal at a time after hormone injection when the levels of normal serum globulins are increased in nonimmunized animals. Serum protein alterations are maximum at the time that the greatest amount of lymphocyte dissolution is occurring and when the most striking lymphopenia is present in the blood. Extention of these experiments indicates that the anamnestic response is probably due to the pituitary-adrenal cortical control of the release of antibody from lymphocytes.[36] This hypothesis would explain why various nonspecific agents produce an anamnestic response[41] and would correlate this response with the simultaneously occurring lymphopenia.

SIGNIFICANCE OF RESULTS

The acute, absolute lymphopenia observed within a few hours after administration of adrenotrophic hormone is the peripheral manifestation of a sudden release of hormones of the adrenal cortex. This is evidenced by depletion of adrenal cholesterol and sudanophilic material shortly after adrenotrophic hormone injection. In addition, the lymphopenia reflects the marked lymphocyte dissolution occurring concomitantly in lymphoid structures and is, therefore, a result of the failure of delivery of normal numbers of lymphocytes to the circulation. Support for this point of view has been obtained in the experiments of Reinhardt and Li[22] who observed a 50 per cent decline in the numbers of lymphocytes in the thoracic duct lymph of the rat within thirty minutes following injection of adrenotrophic hormone. It should be noted that peripheral factors may also be concerned in the removal of lymphocytes, thus contributing to the lymphopenia.[42]

The lymphopenia and augmented serum globulin levels occur at a time when there is maximum dissolution of lymphocytes in the lymphoid structures (six to nine hours). Following this time, restoration of normal lymphoid structure is evident, lymphocyte dissolution ceases, and blood lymphocytes and serum protein values begin to return to normal. At this time there is also a beginning restoration of the adrenal lipids. Within a relatively few hours, therefore, profound physiologic alterations occur as a consequence of a single stimulation of the adrenal cortex and the effects of adrenal cortical steroids on the structure and function of lymphoid tissue. The rate of these physiologic processes is normally under pituitary-adrenal cortical control. This conclusion is further supported by the fact that in adrenalectomized animals there is a lymphocytosis,[20, 34] a diminished rate of lymphocyte dissolution,[43] and a lower

than normal total serum protein level.[16, 33] It should be emphasized that the endocrine mechanism is concerned with regulation of the rates of the processes which have been discussed. It is not to be construed, however, that these physiologic processes cease in the absence of the pituitary or the adrenals. In this respect the physiologic reactions influencing lymphoid tissue structure and function resemble other endocrine-controlled phenomena.

Inasmuch as a variety of unrelated stimuli are known to augment pituitary-adrenal cortical secretion, it might be expected that single exposures of an animal to any one of these stimuli would initiate and reproduce the sequence of alterations which have been described previously. Thus, shortly after the initiation of an infection there is adrenal cortical stimulation,[19, 44-46] an absolute lymphopenia,[47, 48] lymphocyte dissolution and reticulo-endothelial activation,[48, 49] and an increase in the serum globulins.[50-54] A single injection of a variety of toxic chemical agents, such as, arsenic, benzene, antimony, formaldehyde, colchicine, and foreign proteins, activates the adrenal cortex,[19, 43] induces a lymphopenia,[55-59] lymphocytic degeneration,[7, 43, 60-63] and augmented serum globulin levels[43, 64-66] Anesthetics also produce a portion of this sequence of events.[67, 68] Radioactive agents, such as, x-rays, radium, and thorium X, are also effective in causing these alterations.[42, 69-76] Environmental stresses, such as, cold, heat, inanition, trauma, and anoxia, have been shown to reproduce one or more of the series of events under discussion.[7, 19, 43, 77-84] Finally, physiologic agents normally present in the organism, such as, insulin, epinephrine, histamine, pitressin, and thyroid hormone, may, when administered or when present in excess, augment adrenal cortical activity,[19, 43] induce a lymphopenia,[43, 55, 57, 85-87] cause lymphoid tissue dissolution,[7, 10, 43, 87] and in some instances cause an increase in serum globulins.[43, 88]

Most of the many kinds of stimuli previously mentioned have been shown to cause adrenal cortical activation, as demonstrated by histologic or chemical study of the adrenal. Moreover, some of these stimuli are without effect on lymphoid tissue in adrenalectomized animals.[10, 17, 42, 43, 62, 84, 89] The activation of the adrenal cortex is dependent upon the secretion of pituitary adrenotrophic hormone, since certain of these so-called nonspecific stimuli fail to produce any alterations in lymphoid tissue physiology in hypophysectomized animals. It is suggested that a variety of nonspecific influences which have been known for many years to affect lymphoid tissue may be exerting their action through pituitary-adrenal cortical activation.

Many nonspecific stresses may, when applied in large amounts or in great degree, affect lymphoid tissue without hormonal intervention. Thus, it has been shown that high concentrations of benzene will destroy lymphocytes in vitro.[63] The concentration of benzene required, however, was considerably higher than that which could be obtained in the living organism without producing death. Therefore, the dissolution of lymphocytes following benzene injection could not be accounted for on the basis of a direct action of the chemical on lymphocytes. However, the concentration of benzene which is required to produce lymphocyte dissolution in vivo is considerably less than that which will produce similar results in vitro. It is recognized that agents less toxic

systemically than benzene may have a direct destructive action on lymphocytes when administered to experimental animals. Agents of this type which have been shown to be effective in causing thymic involution in adrenalectomized animals when given in large, nonphysiologic doses are desoxycorticosterone, testosterone, estrogens, and diethylstilbesterol.[10, 32, 90] It should be emphasized that most of the data obtained with the use of nonspecific stimuli in relation to lymphoid tissue physiology support the hypothesis that pituitary-adrenal cortical mediation is involved.

A striking example of a stimulus which may exhibit either a direct or an adrenal cortical-mediated effect on lymphoid tissue may be seen from studies with x-rays.[76, 91] The long-recognized effect of x-rays on lymphoid tissue is, in the case of large doses, a direct action on lymphoid tissue, while in small doses, the lymphocytolytic effect of radiation is dependent on pituitary-adrenal cortical mediation.[42, 89]

In 1937, Selye[7] described certain responses, such as, depletion of adrenal cortical lipids, thymic atrophy, gastric hemorrhage, and clouding of the cornea, in experimental animals exposed to a variety of stresses. These reactions were grouped into a syndrome which was termed the "alarm reaction." Selye also noted that repeated exposure to increasing degrees of stress produced an increased degree of resistance against the stress; this phenomenon was called the "adaptation syndrome." The role of the adrenal cortex in the "alarm reaction" was suggested by Selye to be one of protection against histamine which was liberated in augmented amounts during the "alarm reaction." The involution of lymphoid tissue in the stressed animal has now been demonstrated to be due to the augmented secretion of pituitary adrenotrophic hormone.[19] As a result, there occurs an increased release of the steroid hormones of the adrenal cortex, which produce an atrophy of lymphoid tissue.[14, 15, 18] This atrophy is a consequence of the dissolution of lymphocytes which was described previously.

The extensive literature on the role of lymphoid tissue in resistance led us to suggest[33] that the pituitary-adrenal cortical control of lymphoid tissue structure and function integrated the role of the adrenal cortex and the lymphocyte in resistance. One important manifestation of this relationship is the augmented release of antibody from lymphocytes in circumstances of stress.[36, 42] The manner in which various factors play a role in resistance and adaptation is not well understood except in the case of the immune globulins.

Although many of the nonspecific stimuli which have been discussed produce an acute lymphopenia, the blood picture may change subsequently to one of lymphocytosis. Acute lymphocyte dissolution and lymphopenia are shortly followed by restoration of normal lymphocyte numbers in lymphoid structures. The duration of the lymphopenia may be dependent upon the degree to which the adrenal steroids are released, the period over which this accelerated release continues, and the rate of operation of processes concerned with the formation of lymphocytes. Various nonspecific stimuli do not affect the pituitary-adrenal cortical mechanism to the same degree, and, therefore, several types of physiologic manifestations of adrenal cortical activation may be seen.[19] In this con-

nection particular attention must be paid to the role of inanition in effecting involution of lymphoid tissue. Caloric restriction is a potent activator of pituitary-adrenal cortical secretion.[19] The effects of inanition on lymphoid tissue have been shown to be mediated primarily by this endocrine mechanism.[84] In addition, dietary intake will control the supply of materials from which new lymphoid tissue can be formed. It is particularly important to evaluate the inanition factor in nutrition studies designed to elucidate the role of a specific dietary constituent in the growth of lymphoid tissue.

Lymphocyte dissolution may be one of the physiologic stimuli which calls into play factors concerned with the synthesis of lymphocytes. These factors as yet have not been studied adequately. The numbers of circulating lymphocytes in the blood at any time must be a reflection of the balance obtaining among lymphocyte production, lymphocyte dissolution, and peripheral removal of lymphocytes. When the acute effects of the sudden release of adrenal cortical steroids have subsided, the mechanisms concerned with lymphocyte production may manifest themselves to an exaggerated degree. Therefore, the blood picture may alter from lymphopenia to lymphocytosis. The initial lymphopenia produced by most of the nonspecific agents which have been referred to in the previous paragraphs produce lymphocyte dissolution. Therefore, this acute lymphopenia is due to failure of delivery of lymphocytes to the circulation. The subsequent enhanced production of lymphocytes would explain the high level of lymphocytes, following an immediate lymphopenia, which occurs following insulin[87] or epinephrine injection,[86] after low doses of x-rays,[73] and the lymphocytosis in the recovery phase of acute infectious diseases.[92, 93] Blood lymphocyte pictures in disease may be better understood when interpreted in the light of the demonstrated function of lymphoid tissue and its peripheral manifestation, the numbers of circulating lymphocytes.

Many of the same stimuli which have been previously cited as augmentors of pituitary-adrenal cortical secretion, of lymphoid tissue dissolution, of the degree of lymphopenia, and of serum protein levels are also known to increase antibody titers in immunized animals.[1, 41] This enhancement of titer by nonspecific stimuli is known as the anamnestic reaction. These relationships suggested that the anamnestic response was dependent upon the ability of these agents to increase adrenal cortical hormone secretion. Data obtained[36, 42, 89] establish clearly that an enhancement of antibody titer occurred when adrenal cortical preparations were administered to previously immunized animals having no circulating antibody. Adrenotrophic hormone was highly active in the normal but not in the adrenalectomized animal. Certain toxic agents, potassium arsenite, benzene, and low doses of x-rays produced the anamnestic reaction in normal animals. These agents were without effect in the absence of the adrenals. All of the adrenalectomized animals had demonstrable antibodies in extracts of lymphocytes from their lymphoid tissue. The pituitary-adrenal cortical mechanism is essential for the release of antibodies from lymphocytes except under those conditions in which there is a direct effect of the toxic agent on lymphocytes, such as high dose of x-rays in adrenalectomized animals.[42, 89]

The role of the adrenal cortex in controlling the rate of release of lymphocyte constituents correlates the functions of the adrenal and of the lymphocyte in resistance. The participation of the adrenal cortex is dependent upon its stimulation by pituitary adrenotrophic hormone.

CONCLUDING REMARKS

In the opening statements of this presentation, attention was called to the paucity of knowledge concerning the fate and function of the lymphocyte and the physiologic control of lymphoid tissue. One of the fates of the lymphocyte elements is a dissolution of many of these cells in the lymphoid structures. This dissolution often results in the disintegration and removal of the structural material of these cells. Some lymphocytes may shed portions of their cytoplasm into the surrounding lymph without death of the residual nuclei; under these circumstances, regeneration of normal amounts of cytoplasm may follow. The cytoplasm yielded to the lymph by the lymphocyte contains gamma globulin and, in the immunized animal, antibody. Gamma globulin is found within lymphoid structures, is present in lymphocytes, and its rate of release from these cells is under control of pituitary-adrenal cortical secretion. As a consequence of this hormonal control of the rate of lymphocyte dissolution, the numbers of lymphocytes in the blood and in lymphoid tissues is influenced by the degree of adrenal cortical activity.

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PURPURA FOLLOWING ESTROGEN THERAPY, WITH PARTICULAR REFERENCE TO HYPERSENSITIVITY TO (DIETHYL) STILBESTROL AND WITH A NOTE ON THE POSSIBLE RELATIONSHIP OF PURPURA TO ENDOGENOUS ESTROGENS

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THE literature indicates that the administration of estrogenic substances, and particularly stilbestrol, while at times productive of nausea and vomiting, does not cause serious toxicity in human beings. Thus, Shorr and coworkers,[1] Davis,[2] Davis and Boynton,[3] and MacBryde and associates[4] made careful observations of the blood in large series of women receiving 1 to 5 mg. of stilbesterol daily over long periods of time; they failed to find any significant changes in the blood or any evidence of a hemorrhagic tendency. The study of Karnaky[5] seems even more convincing in this respect. He gave remarkably large doses of stilbestrol to 136 women and eighty-six children. The adults received as much as 500 mg. at a single dose, 250 mg. once or twice weekly, or 100 mg. daily. The majority of the children were given 1 to 5 mg. daily; eight, however, received 10 to 25 mg. per day or every other day for periods of one to eight months. Karnaky relied on the sedimentation rate and complete blood counts to detect toxic effects. None were observed, and he therefore concluded that stilbestrol is entirely safe. Nevertheless, certain cases have been reported in the literature which indicate that occasional individuals may exhibit an idiosyncrasy or sensitivity to stilbestrol or other estrogenic substances, with resultant injury of the liver or bone marrow. Loftis[6] reported the case of a 44-year-old white woman who developed purpura over the lower extremities and bleeding from the gums while receiving weekly injections of an estrogen. The purpura disappeared within three weeks after the drug was stopped. Repeated trials with varying dosages always caused recurrence of purpura. Laboratory studies, including platelet counts, revealed nothing abnormal. A bone marrow examination was not reported. Chevalier and Umdenstock[7] observed a case of fatal myelophthisis following injections of large amounts of estradiol benzoate. This patient had received estradiol repeatedly over a period of ten years because of recurrent pruritus vulvae. The last course consisted of twenty injections of 10 mg. each in a two-week period. Shortly thereafter, the patient developed a profound anemia, leucopenia of 800 cells per cubic millimeter with 30 per cent neutrophiles, and an outspoken purpura with 56,000 platelets per cubic millimeter. Herbst[8] observed purpura in two men receiving estradiol propionate for cancer of the prostate. There was no reduction of platelets in either case, and it was stated that the purpura was controlled by administration of calcium gluconate.

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Instances of probable hepatotoxic effect of stilbestrol in human beings have been reported by Elias and Schwimmer[9] and Seligman.[10] The former case is of special interest in that the authors were able to demonstrate a distinct allergy to stilbestrol in their patient. They believed that the hepatocellular impairment was allergic in character. Jaundice was of relatively short duration and recovery was apparently complete.

A considerable literature has accumulated with respect to the effect of stilbestrol and other estrogens on the blood and blood-forming organs and the liver, in animal experiments. It is evident from these studies that there is a distinct difference in the sensitivity of different species. Arnold[11] showed that daily intramuscular injections of 4 to 10 mg. of stilbestrol in dogs commonly produced severe aplastic anemia and thrombocytopenic purpura. Tyslowitz and Dingemanse[12] found that dogs tolerated 1 mg. of estradiol or stilbestrol daily during a period of nine months without exhibiting any significant changes in the blood, but if given 5 mg. of either estrogen daily, a prompt fall in all blood elements occurred within one to three weeks after the injections were begun. The erythrocyte count and hemoglobin fell rapidly before any evidence of hemorrhage was noted. The platelets then dropped to very low levels and severe purpura occurred. The leucocytes showed an initial rise followed by a marked decrease in the number of granulocytes. Bone marrow studies in these dogs revealed a marked hyperplasia involving all of the cellular elements. These investigators[12] concluded that the estrogen caused a primary injury to the bone marrow followed by changes in the peripheral blood. Their results were confirmed by MacBryde and co-workers[4] and Crafts.[13] Castrodale and associates[14] compared the effect of stilbestrol and estradiol in dogs, using both parenteral and oral routes of administration, and found that thrombocytopenia and hemorrhage occurred sooner and that the survival time was shorter in those animals receiving estradiol. The onset of the blood changes and of purpura was delayed in the animals receiving the estrogen orally, but the severity was just as great after their appearance. They also noted that the bone marrow initially showed an increase of the myeloid elements with decreased areas of erythropoiesis, followed by a generalized hypoplasia. The megakaryocytes were markedly decreased early in the course of the experiment. The effect of stilbestrol in rats is evidently much less marked. A mild decrease in platelets early in the course of experiments in which large amounts of stilbestrol were injected was observed by von Haam and co-workers,[15] but there was no purpura nor any significant change in the leucocytes. The rat bone marrows, however, were markedly hyperplastic. Page and associates[16] implanted pellets of stilbestrol subcutaneously in rats and failed to notice any subsequent changes in the blood or bone marrow. Jacobson[17] injected relatively large doses of estradiol in white mice and found a moderate decrease in hemoglobin and red blood cells in the peripheral blood. He noted that the bone marrow of the mice showed a marked replacement of the marrow spaces by endostial new bone formation, the remaining areas of active marrow being hyperplastic. Crafts[13] and Tyslowitz and Hartman[18] were unable to produce any significant changes in the

blood or bone marrow of Rhesus monkeys, in spite of the administration of large amounts of estradiol or stilbestrol.

The effects of estrogens on the liver in animals has not been as extensively studied. Castrodale and associates[14] noted fatty metamorphosis and focal necrosis in the livers of many of their series of dogs receiving relatively large amounts of stilbestrol or estradiol, either orally or parenterally. Roberts and co-workers[19] later reported that large doses of estrone or estradiol benzoate produced changes in the liver, bone marrow, and peripheral blood of dogs, which were indistinguishable from those produced by stilbestrol. Hepatic changes consisted of fatty metamorphosis and hydropic degeneration.

It should be emphasized that in all of the experimental work which has been mentioned, the amounts of estrogenic substance necessary to produce evidence of injury were greatly in excess of the therapeutic dose in human beings. The purpose of the present report is to describe five cases in which purpura was first noted following estrogenic therapy. Four of these were in women, one was in a man. The latter individual also exhibited marked evidence of hepatic functional impairment.

CASE REPORTS

CASE 1.*—E. S., a 55-year-old housewife, was first admitted to the University Hospital in March, 1943, because of weakness, menorrhagia, and purpura.

The patient had noted the onset of the menopause in the fall of 1936; it was characterized by hot flashes, increasing nervousness, weakness, and cessation of menses. In 1937 she consulted a local physician who gave her several intramuscular injections of amniotin in oil. She had little improvement of the symptoms and in June, 1939, consulted another physician. At this time intensive estrogen therapy was begun, and from June, 1939, until March, 1943, she received many intramuscular injections of estrone and estradiol. It has not been possible to determine the exact amount. In September, 1941, in addition, oral stilbestrol therapy was commenced. The patient received from 1 to 1.5 mg. of stilbestrol daily until March, 1943. In June, 1942, the patient was seen by the physician because of an episode of uterine bleeding which lasted three days. At this time she also complained of bruising very easily. The hemoglobin was found to be 80 per cent; the red blood cells were 4,000,000 per cubic millimeter; the leucocytes, 2,400 per cubic millimeter; and the platelets, 15,000 per cubic millimeter. Bleeding time by the Duke method was 15 minutes. The patient was treated with several blood transfusions, vitamin K, liver extract, and pectin, but moderate uterine bleeding lasted three to four days and recurred regularly each month. She continued to bruise easily on slight trauma.

In December, 1942, the patient first noticed petechiae over the legs, forearms, and chest. In February, 1943, there was a profuse uterine hemorrhage, and she was admitted to a private hospital. The examination of the blood at this time showed the hemoglobin to be 60 per cent; the red blood cells were 3,000,000 per cubic millimeter; the leucocytes, 2,900 per cubic millimeter; and the platelets, 8,700 per cubic millimeter. During this period of hospitalization the patient was given several blood transfusions and five deep x-ray treatments to the ovaries. She was discharged after nine hospital days. Uterine bleeding stopped on March 15, 1943, after four weeks of continuous rather severe hemorrhage, but the purpura persisted, and on March 25, 1943, she was admitted to the University of Minnesota Hospital. It should be noted that the patient had continued to take stilbestrol up to this time.

The past history revealed nothing of significance. The catamenia had been entirely normal prior to the menopause, and there was no previous indication of a hemorrhagic tendency.

*This case was studied through the courtesy of Dr. H. W. Quist, Minneapolis, Minn.

Physical examination revealed numerous petechiae over the body and several large ecchymoses over the patient's legs, chest, and forearms. The spleen was barely palpable on deep inspiration. There were no other findings of significance.

The hemoglobin was 10.8 grams per 100 c.c.; the leucocytes were 2,000 per cubic millimeter; the reticulocytes, 1.5 per cent; and the platelets, 160,000 per cubic millimeter. The bleeding time by the Duke method was 42 minutes; the prothrombin time was normal. The cephalin-cholesterol flocculation test was 3 plus at 24 hours. A sternal bone marrow puncture revealed generalized hypoplasia of all the cellular elements of the bone marrow. Megakaryocytes were very scarce and showed degenerative changes.

All estrogenic therapy was stopped. The patient was given several blood transfusions, and was discharged in relatively good condition. It is noteworthy that she was complaining rather severely of hot flashes and that she wished very much to take additional stillbestrol for relief. She was advised strongly not to do this.

The purpura gradually disappeared, and the patient was relatively free of hemorrhages so far as can be determined from April, 1943, until July, 1944, when petechiae and ecchymoses again appeared over the legs, arms, and chest. It may be noted, however, that the platelets were repeatedly found to be reduced during this interval: 2,200 on April 16, 1943; 38,000 on Aug. 24, 1943; 6,400 on Oct. 18, 1943; and 148,000 on Feb. 10, 1944. The uterine bleeding did not recur. In September, 1944, she was given twelve roentgen treatments of ten minutes each to the ovaries, a total of 3,474 r.; the purpura persisted, however, and the patient was readmitted to the University Hospital in December, 1944. Physical examination at this time was essentially negative except for marked purpura over the legs, forearms, and chest. The hemoglobin was 11.5 grams; the leucocytes were 2,050 per cubic millimeter with 62 per cent neutrophiles. The platelets were 62,000 per cubic millimeter. The prothrombin time was again normal and cephalin-cholesterol flocculation test at this time was negative. The bleeding time was markedly increased. Several sternal bone marrow punctures were attempted, but no marrow could be aspirated. After four days in the hospital, the patient was discharged and has been followed in the Out-patient Clinic until the present time. She has persisted in having purpura of moderate degree, and the platelets have varied on a number of occasions between 30,000 and 120,000 per cubic millimeter. The leucocytes have varied between 1,800 and 3,100 with 50 to 65 per cent neutrophiles. The hemoglobin ranges between 10.5 and 12.5 grams per 100 c.c., and the red blood cells range between 3.8 and 4.5 million per cubic millimeter. Trial of various substances has been made, including refined liver extracts by intramuscular injection, whole desiccated liver by mouth, and folic acid by injection in amounts ranging up to 50 mg. per day. None of these has had any demonstrable effect.

This patient, then, has a chronic thrombocytopenia purpura and leucopenia, which is remarkably stable at a level permitting a reasonable degree of activity but which, nevertheless, interferes with her life to a considerable extent and threatens at any time to become more severe.

CASE 2.—Y. T., a 53-year-old housewife, was admitted to the University Hospital in December, 1944, because of purpura over the arms, legs, and chest.

The patient had the onset of the menopause in January, 1942, when the menses became irregular and profuse and she began to have increased nervousness, weakness, and hot flashes. In May, 1942, she had an episode of severe menorrhagia. Hysterectomy and bilateral salpingo-oophorectomy were performed at a private hospital, and at this time, May, 1942, oral stilbestrol therapy of one tablet daily was begun and continued until November, 1944. In November, after two and one-half years of continuous stilbestrol therapy, the patient first noticed petechiae and ecchymoses over the arms, legs, and chest. These persisted and she entered the University of Minnesota Hospital on Dec. 13, 1944. The past history revealed nothing of significance other than a mild arthritis of the cervical spine for which intravenous injections of streptococcus vaccine were given during October and November, 1944. This will be considered again in the following.

The physical examination revealed nothing of significance other than many petechiae and several ecchymoses over the legs, arms, and chest.

The hemoglobin was 10.3 grams; the red blood cells were 4,000,000 per cubic millimeter; the leucocytes, 5,250 with 57 per cent neutrophiles; and the platelets, 70,000 per cubic millimeter. The cuff test was negative. The bleeding time by the Duke method was 16 minutes. A platelet count performed one week later was 90,000 per cubic millimeter. Stilbestrol therapy was stopped when the patient entered the hospital. The purpura rapidly improved, and after three days she was discharged to the Outpatient Clinic where she has been followed to the present time. Within two weeks from the time of discharge the petechiae had entirely disappeared. On Dec. 26, 1944, the platelet count was 180,000 per cubic millimeter; on Jan. 2, 1945, 210,000; and on March 14, 1945, 180,000. The sternal bone marrow obtained during the hospital admission revealed a marked hyperplasia involving chiefly the granulocytes. The megakaryocytes were also increased in number. The bromsulfalein test (5 mg. per kilo at 45 minutes) was negative but the cephalin-cholesterol flocculation test was 3 plus in 48 hours. The urine urobilinogen was normal.

There was no recurrence of purpura until March 1, 1947. Shortly before this, the patient had taken benzestrol[19] because of persistent hot flashes. The onset of purpura was noted three days after the benzestrol was started. The drug was discontinued at once and after four days the purpura began to disappear. By March 25, it was no longer evident. On March 11 when it was still widespread, the platelets numbered 165,000 per cubic millimeter and the cuff test was strongly positive.

Streptococcus vaccine therapy was resumed in 1945 and has been continued irregularly until the present time. There has been no evidence of any recurrence of the purpura except on the one occasion following benzestrol. Thus, it appears improbable that the vaccine had any relation to the thrombocytopenia.

CASE 3.*—S. G., a 23-year-old housewife, had been well except for irregularity of the menstrual periods. The interval had always been greater than thirty days, and since her marriage in 1943, the periods were even more irregular, there having been not more than eight or ten periods in the two years. On Feb. 15, 1945, because of not having had a period since October, 1944, the patient consulted a physician who gave her ½ c.c. benzestrol.[19] This dose was repeated on Feb. 21 and 22 and on March 6. On April 4, she was given 2.5 mg. of dimenformin benzoate, and on April 5, 12.5 mg. of trigestin. One week later menstruation commenced and lasted for five days. Two days after termination of the period a "rash broke out all over her body." She noticed that it was worse on the legs and hands but that it was present everywhere to at least a slight degree. She also noted a few bruises on various parts of the body.

The past history did not reveal anything of significance. The family history was of interest in that a sister died at the age of 23 of leucemia. The patient has two brothers and one sister living and well. Her parents are also well.

She has had occasional headaches, for which she takes a tablet composed of acetophenetidine, aspirin, and caffeine. She has taken one of these about every two weeks since October, 1944.

The physical examination revealed nothing other than a diffuse purpura. Multiple petechiae were present with increasing frequency toward the distal portions of the extremities, most marked on the lower legs and ankles. However, there were a few on the face, chest, abdomen, arms, hands, and thighs. The liver and spleen were not palpable.

The hemoglobin was 13.7 grams; the red cells were 4,150,000; the leucocytes, 7,200 per cubic millimeter with 61 per cent neutrophiles; the platelets, 12,100; the bleeding time by the Duke method 24 minutes. A repeat platelet count was 20,000; the prothrombin was 100 per cent of normal. On April 25, there was no evidence of further spread of the purpura, but the platelets were only 11,000. On June 6, the platelet count was 13,200, the bleeding time 23 minutes. The bone marrow obtained by sternal puncture was said to reveal nothing abnormal. On July 27, 1945, the platelet count was 33,000, and on August 9, 40,000. At this time the purpura had almost entirely disappeared. On November 13, a few new petechiae

*This case is reported through the courtesy of Dr. J. Garrott Allen of the Department of Surgery and the Metallurgical Laboratory, University of Chicago.

were observed. The irregular menstruation with long intervals between the periods has continued. The platelet count on Nov. 13, 1945, was 12,000. On Jan. 2, 1946, the patient was seen because of recurrence of petechiae in moderate number, especially on the legs. The patient was last observed on Feb. 16, 1946. At this time the hemoglobin was 10.9 grams; the red blood cells were 3,880,000; the leucocytes, 5,700 with 62 per cent neutrophiles; the platelets, 15,500. Only a few petechiae were noted.

CASE 4.*—M. C. was a 79-year-old retired businessman. In 1939, at the time of transurethral resection, it was found that the patient had a carcinoma of the prostate. Stilbestrol was commenced on July 3, 1942, 0.5 mg. daily. From Nov. 17, 1943, until shortly before admission to the hospital, he took, somewhat intermittently, 3 mg. daily. The breasts had become distinctly enlarged. From time to time during this period the patient had received small amounts of sulfadiazine because of a urinary tract infection. He had not, however, received any sulfadiazine for three months prior to death and for one month prior to the first appearance of purpura. Ecchymoses first became evident on the extremities and neck about Feb. 25, 1946. Because of increasing purpura with some very large ecchymoses, particularly over the abdomen, the patient was admitted to the hospital on March 10, 1946. The hemorrhages were in the form of large ecchymoses and even considerable extravasations of blood under the skin. One massive extravasation was noted over the lower abdomen extending down into the pubic area and including the scrotum, also down onto the thighs. There were numerous ecchymoses elsewhere but small petechiae were very rare. There was no jaundice at the time of admission. The liver and spleen were not palpable, and there was no lymphadenopathy. There were no evidences of metastases to the bones. (X-rays of spine and pelvis were negative.) The platelet count was 40,000 on the day following admission. On the next day it was found to be 70,000. The prothrombin time was 17 per cent of normal. The serum albumin was 2.6 grams per cent; the serum globulin, 2.3. The cephalin-cholesterol flocculation test was 3 plus in 24 hours. The urine urobilinogen was 42 mg. in 24 hours (normally not above 3.5 mg.). The later course in the hospital was characterized by retinal hemorrhages, hemoptysis, and gastrointestinal bleeding, in addition to further bleeding into the skin. Coincident with transfusions and vitamin K, the prothrombin time came up to 80 per cent of normal, following which there was a temporary cessation of hemorrhages. Later, however, in spite of continued vitamin K administration, the prothrombin time dropped to 30 per cent and the generalized hemorrhagic tendency recurred. During the last six weeks in the hospital, the platelet count gradually rose to 150,000, but the bleeding persisted. An additional feature of interest that was not explained was a marked hypocalcemia with tetany. The serum calcium was 6.8, and on frequent occasions there was manifest tetany requiring injection of calcium. Death was believed to be due to anemia and exhaustion. Slight jaundice was observed in the last ten days of life. There was no evidence of steatorrhea.

Necropsy in this case revealed very little. The prostate was small and rather soft but exhibited definite adenocarcinoma microscopically. The liver was described as being "granular and firm," but not enlarged. Microscopically the sections of the liver revealed nothing other than mild passive congestion. The bone marrow was not examined.

CASE 5.†—H. G. was a 45-year-old housewife. Menstruation first became irregular in February, 1942. There was no period in May, 1942, and again from July to October, although during this interval one brief period of rather profuse bleeding for two hours occurred. In January, 1943, the patient was overemotional, had swelling of the hands, tingling of the skin, chills, sweats, and hot flashes. She was given 0.5 mg. of stilbestrol daily for one week, together with nembutal, ½ grain three times daily. She was seen two weeks later, at which time the nervousness and exhaustion, and also the hot flashes and sweats, were considerably improved. Stilbestrol in an amount of 0.5 mg. daily was continued for one week. From this time on the stilbestrol was given intermittently in the same dose for

*This case was seen in consultation with Dr. D. P. Head to whom the authors acknowledge their gratitude for permission to report the findings which are given.

†This case was studied through the courtesy of Dr. Henry L. Ulrich, Minneapolis, Minn.

a period of four months. In August, 1944, when the patient was next seen, perspiration was marked and there were frequent dizzy spells, particularly on waking up in the morning. Stilbestrol was resumed in a dose of 0.2 mg. daily. She was not seen again until March, 1945. The same dose, namely, 0.2 mg., was prescribed again. The patient was also given calcium lactate, 5 grains three times a day, and ½ grain of nembutal three times daily. She was next seen in June, 1945, and stilbestrol was given again, 0.2 mg. daily. In August she was given 2,000 units of estrone hypodermically. This dose was repeated in November, 1945, the stilbestrol being taken intermittently in the interval. Bleeding was first noted in June, 1946. This was from the gums and uterus. From this time on there has been recurrent uterine bleeding, also intermittent hemorrhage from the gums and nose, and purpura of the skin. Numerous petechiae and ecchymoses have been noted on a number of occasions.

The physical examination revealed nothing of significance in other respects. The spleen and liver were not palpable and there was no lymphadenopathy.

Blood examination on June 6, 1946, revealed the following: Hemoglobin 80 per cent, red blood cells 4,430,000, leucocytes, 3,700, 49 per cent neutrophiles, 36 per cent lymphocytes, 11 per cent monocytes, 3 per cent eosinophiles, and 1 per cent basophiles. On Sept. 24, 1946, the hemoglobin was 14.5 grams; the platelet count, 121,000 per cubic millimeter; the bleeding time, 5 minutes, 2 seconds (Duke method); the cuff test, markedly positive, 45 petechiae in a circle 1 inch in diameter on the forearm; the prothrombin time by the Quick method, 12.7 seconds (control, 12.4). Since this initial study, frequent platelet counts, bleeding times, and cuff tests have been carried out with essentially the same findings, regardless of the degree of purpura exhibited by the patient at the particular time. So far as is known she has not received any estrogen therapy since June, 1946. The purpura has not been alarming up to the present time, and the patient has been able to continue her activities on a limited scale.

STUDIES OF SKIN SENSITIVITY TO ESTROGENIC SUBSTANCES

Three of the previously described patients (Cases 1, 2, and 5) were available for skin tests to determine possible sensitivity to estrone and stilbestrol. Twenty-five individuals, either normal or suffering from various diseases, served as controls. Sixteen of these were tested for sensitivity to estrone, the remainder to stilbestrol. In each instance the test consisted of injecting intradermally 0.1 c.c. of a 0.2 per cent suspension in physiological saline. The same amount of the vehicle alone was injected in the opposite arm as a control. Observations of reactions were made at 30 minutes, 1 and 4 hours, and 24 hours, as noted in the following. The results of the tests are given in Table I. It is seen that Cases 1 and 2 exhibited distinct skin sensitivity to stilbestrol, while Cases 2 and 5 were sensitive to estrone. In none of these three cases was there any sensitivity to the vehicle. The reactions in each instance were of erythematous type, only slightly raised and without definite whealing. Their appearance suggested the presence of an active capillary or arteriolar hyperemia without transudation. Two of the control cases exhibited sensitivity to the estrone solution and one of these was positive to the solution of stilbestrol, but both showed equally positive reactions to the vehicle, so they cannot be classified as positive to the estrogens. Thus, it is evident that the only positive reactors among the twenty-eight individuals were the three with purpura, Cases 1, 2, and 5 of the present series. So far as could be determined from the records of the control individuals none had at any time received estrogen therapy.

Prausnitz-Küstner passive transfer was attempted in Cases 2 and 5 with estrone and in Cases 1 and 2 with stilbestrol, but this was uniformly negative.

TABLE I. SKIN SENSITIVITY TO STILBESTROL AND ESTRONE

CASES IN PRESENT SERIES	CONTROL TEST (0.1 C.C. NORMAL SALINE)				ESTRONS				STILBESTROL		
	30 MIN.	1 HR.	4 HR.	24 HR.	30 MIN.	1 HR.	4 HR.	24 HR.	30 MIN.	1 HR.	4 HR.
1	0	0	0	0	0	0	0	0	1×1 cm.	1×1 cm.	0
2	0	0	0	0	3×2.5 cm.	3×2.5 cm.	0	0	2×2 cm.	1×1 cm.	0
5	0	0	0	0		3×2.5 cm.	0	0	0	0	0

CONTROL CASES												
NO.	AGE	SEX	DIAGNOSIS									
1	75	F	Cholecystitis	0	0	0	0	0	0	0	0			
2	45	F	Hyperthyroidism	0	0	0	0	0	0	0	0	0	0	0
3	67	F	Typhoid carrier	0	0	0	0	0	0	0	0			
4	66	F	Irritable colon	3×3 cm.	3×3 cm.	0	0	3×3 cm.	3×3 cm.	0	0	0	0	0
5	41	F	Pernicious anemia	2×2 cm.	2×2 cm.	0	0	2×2 cm.	2×2 cm.	0	0	2×2 cm	0	0
6	46	F	Leucemia	0	0	0	0	0	0	0	0	0	0	0
7	61	F	Hyperthyroidism	0	0	0	0	0	0	0	0			
8	53	F	Cardiac failure	0	0	0	0	0	0	0	0	0	0	0
9	60	F	Duodenal ulcer	0	0	0	0	0	0	0	0			
10	54	F	Pneumonia	0	0	0	0	0	0	0	0			
11	51	F	Hypertension	0	0	0	0	0	0	0	0	0	0	0
12	66	F	Normal	0	0	0	0	0	0	0	0			
13	28	F	Normal	0	0	0	0	0	0	0	0	0	0	0
14	31	M	Normal	0	0	0	0	0	0	0	0	0	0	0
15	71	F	Carcinoma of colon	0	0	0	0	0	0	0	0	0	0	0
16	55	F	Cardiac failure	0	0	0	0	0	0	0	0			
17	42	F	Jaundice	0	0	0	0					0	0	0
18	57	F	Cholecystitis	0	0	0	0					0	0	0
19	56	F	Hypertension	0	0	0	0					0	0	0
20	39	F	Thrombophlebitis	0	0	0	0					0	0	0
21	28	F	Brucellosis	0	0	0	0					0	0	0
22	73	F	Arteriosclerosis	0	0	0	0					0	0	0
23	25	F	Normal	0	0	0	0					0	0	0
24	25	F	Normal	0	0	0	0					0	0	0
25	23	F	Normal	0	0	0	0					0	0	0

Thus, it is questionable whether the skin reactions which were noted were due to circulating antibody. Precipitin tests were not carried out due to the insolubility of stilbestrol and estrone in water. The nature of these skin reactions is not understood but it seems likely that their significance is fundamentally related to the clinical manifestations in these cases, especially in view of the relatively large number of negative controls. The possibility of a specific tissue sensitivity is considered.

DISCUSSION

While the cases described in the foregoing cannot be accepted as proving a causal relationship between stilbestrol or other estrogenic substances and purpura, they do, nevertheless, suggest that such a relationship may at times exist, and they point to the need of greater conservatism in the administration of these substances. They also indicate the desirability of frequent observations with respect to the possible appearance of a hemorrhagic tendency in patients receiving continued estrogenic therapy. It is believed justified, in view of the present results of the skin sensitivity studies, to skin test patients in advance of the prescribing of estrogenic substances. In this connection, however, we recognize that the sensitivity shown by the present cases may have been acquired rather than inherent. In any event the appearance of signs of bone marrow or capillary injury, or of hepatic functional impairment, would appear to warrant discontinuance of estrogen therapy and the application of skin tests to determine possible sensitivity to estrogenic substances. It is clear that a relationship in these cases would have to be regarded as an idiosyncrasy or hypersensitivity, possibly analogous to the occasional case of neutropenia or agranulocytosis which follows amidopyrine, or the thrombocytopenic purpura at times seen following sedormid therapy, and concerning which a number of papers have appeared in the literature. Evidence as to the usually innocuous character of stilbestrol was cited at the outset. The onset of purpura following the exhibition of stilbestrol and its disappearance and reappearance after discontinuance and readministration, as in Case 2 of the present series and in the case reported by Loftis,[6] lend further support to the belief in a causal relationship. The regular production of thrombocytopenic purpura in dogs with amounts of stilbestrol larger than those commonly used in human therapy is quite compatible with the concept that the occasional cases of purpura noted in human beings after stilbestrol therapy represent idiosyncrasies or hypersensitivity to the drug. The possibility cannot be excluded that the purpura in Case 3 was related to the occasional ingestion of acetophenetidin.

It is also of interest that a number of papers have called attention to the association of purpura with menstruation.[21-24, 26, 28-30] In these studies it was at times noted that the platelet counts were normal between the periods, only to decline at the beginning of each menstruation and with the onset of purpura. With the cessation of menstruation, the platelet counts returned to normal and the purpura disappeared. Nagy[22] reported two cases of thrombocytopenic purpura in which irradiation of the ovaries was followed by an increase of platelets with disappearance of purpura and menorrhagia. For these instances,

Nagy suggested the term "purpura dysovarica." As a matter of fact, it was chiefly because of Nagy's report that radiation was given to the ovaries in Case 1 of the present series. No apparent benefit was noted, but it is quite likely that the bone marrow injury in this instance was exogenous while it would appear to have been endogenous in Nagy's cases. In this connection, attention may be called to a case reported by Leschke and Wittkower.[24] A 23-year-old woman had had severe menorrhagia since onset of the menses at the age of 11. When the patient reached the age of 22, the platelets were found to be 3700 per cubic millimeter. The surgeon removed the uterus and a cystic right ovary. This was followed by prompt cessation of the hemorrhagic tendency, and for two years there was no difficulty, after which time, however, the purpura recurred.

The relative frequency of thrombocytopenic purpura in women after puberty has been stressed repeatedly.[26, 28] Purpura is relatively common during pregnancy and has often constituted a severe complication. A number of the cases collected from the literature by Rushmore[27] exhibited purpura only during pregnancy, and some of them had a recurrence of purpura in subsequent pregnancies. On the other hand, Evans and Perry[28] observed a case in which a thrombocytopenic purpura disappeared entirely during pregnancy, only to reappear after delivery.

There is disagreement as to the platelet counts of normal women during their menstrual cycles, Genell[31] and Pohle[32] having reported a noticeable decrease in the platelet count at the beginning of each period and Damashek[33] having found just the opposite. David[23] emphasized the occurrence of hemorrhages and of positive Rumpel-Leede (capillary fragility) tests during menstruation. This question obviously deserves further study.

The possibility of sensitivity to endogenous estrogen, as suggested by the foregoing, is supported in some measure by the fact that two of the cases in the present series exhibited skin sensitivity to estrone, a natural estrogenic substance. One might assume that in certain individuals, a tissue, perhaps a capillary sensitivity, if not inherent, becomes established as times goes on. Evidence is lacking as to the nature of this sensitivity and the manner in which it might operate to produce purpura. The concept of sensitivity to endogenous estrogen has been elaborated by Zondek[34, 35] under the general appellation of "endocrine allergy." Zondek has demonstrated skin sensitivity to natural estrogens in a variety of pathologic states and has shown that desensitization is frequently beneficial.

The possibility of hepatotoxic effect of stilbestrol is suggested both by the animal experiments and the human cases previously mentioned.[9, 10] In Case 2 of the present group the cephalin-cholestrol flocculation test was positive in association with the thrombocytopenic purpura and negative after discontinuance of stilbestrol and disappearance of the purpura. The possibility exists, however, that there was a mild liver injury secondary to the purpura and unrelated to the stilbestrol. Case 4 of the present series was of particular interest because of the association of a severe hemorrhagic state with thrombocytopenia and hypoprothrombinemia, together with other evidence of liver

functional impairment following long continued use of stilbestrol for carcinoma of the prostate. The autopsy findings in this case did not indicate that the hemorrhagic state was related either to the carcinoma or to any demonstrable liver disease of ordinary type.

SUMMARY AND CONCLUSIONS

1. Five cases are reported in which purpura followed the administration of stilbestrol or other estrogenic substances. Four of these were in women being treated for menopausal symptoms or amenorrhea; one was in an elderly man with carcinoma of the prostate, without evidence of metastases.

2. In four of these cases the estrogen therapy, mainly stilbestrol, had been given for long periods, ranging from two and one-half to seven years. In one of these four, however, there were long intervals during a total of three years, in which none was given. In the fifth case estrogens were given parenterally, intermittently over a two-month period.

3. The purpose of reporting these five cases is simply to call attention to the possibility of a causal relationship between stilbestrol and other estrogens and purpura. Evidence of hypersensitivity to stilbestrol or estrone or both, was manifested in each of the three cases available for skin testing, although absent in twenty-five control individuals. Circulating antibody could not be demonstrated by passive transfer. Nevertheless, the possibility must be considered that hypersensitivity to exogenous or endogenous estrogens may at times constitute the major factor in the production of purpura.

4. The literature relating to estrogenic substances is discussed from the following standpoints: (a) The reported cases of supposed toxic effect in human beings, in which abnormalities related to the liver or blood were noted; (b) the experimental production of purpura and hepatic injury in animals by means of their administration; and (c) the question of effect of endogenous estrogen in the production of purpura.

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ESSENTIAL THROMBOCYTOPENIC PURPURA

Autopsy Findings in Thirty-Six Cases

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THE files of the pathology department of the University of Minnesota contain the records of over 51,000 autopsies. Most of the patients with purpura listed are secondary types following such diseases as leukemia, meningococcic sepsis, bacterial endocarditis, scarlet fever, measles. Some of them are examples of drug idiosyncrasies. After eliminating these cases, there remained thirty-six that belonged to the essential or idiopathic group. Wiseman, Doan, and Wilson[1] and Wintrobe[2] have given rigid criteria for the definition of essential thrombocytopenic purpura from the clinical and anatomical viewpoint.

The average age of these thirty-six patients was 30.5 years. Women predominated, twenty-four to twelve men. This confirms the findings in the literature that essential thrombocytopenic purpura is primarily a disease of young people and that the women show a marked preponderance. It is possible that the physiologic decrease in the number of platelets during menstruation may be a predisposing factor for the development of the signs of purpura in women when associated with other causative factors that decrease the platelet level. Hormones may be another factor. The average platelet count in these cases was 55,000 per cubic millimeter. The bleeding times varied from ten minutes to four hours, the coagulation time from three to eight minutes.

The gross findings at autopsy, apart from the hemorrhages that have resulted in the death of the patient, are meager. The body may have the anemic appearance that follows profuse or prolonged hemorrhage. The degree of anemia may be very severe. The hemorrhages may be so widespread that it is difficult to consider any part of the body as the principal site of the bleeding. There were twelve such cases in the total number of thirty-six. In twelve of these death was due to intracranial hemorrhages. Unconsciousness and death may occur within a short time after the onset of acute purpura. Most of the hemorrhages were located beneath the dura mater and were diffusely scattered over the surfaces of the brain. At times it was difficult to decide whether the hemorrhage was extrinsic or intrinsic to the arachnoid membrane. The fact that some of the hemorrhages had the appearance of a subdural hematoma with a tendency towards organization of the clot to the inner surface of the dura would tend to show that most of the hemorrhages are subdural rather than subarachnoid in origin. Occasionally intracerebral hemorrhages may occur with or without an associated subdural hemorrhage. The intracerebral and intracerebellar hemorrhage may vary from petechiae to massive hemorrhages. Once the diagnosis of essential thrombocytopenic purpura is established the

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risk of death from fatal hemorrhage is probably higher than the operative mortality of a splenectomy in the hands of capable surgeons.

In two cases bleeding from the gastrointestinal tract predominated. The hemorrhages varied from massive ecchymosis to petechiae involving the mucosa, walls, and serosa of the stomach, intestine, and colon. In one case the principal bleeding was from the urinary tract. The bleeding was largely from the mucosa of the renal pelvis and bladder. Large perirenal hematomas were not uncommon in other cases. Nine patients continued to suffer from widespread hemorrhages following splenectomies. In one patient, who died after a splenectomy, an accessory spleen that weighed 12 grams[3] was found at autopsy.

The earlier investigators centered their interest largely upon the spleen. The normal adult spleen weighs approximately 150 grams. The average weight of twenty spleens in patients over 20 years of age in this series was 232 grams. The smallest weighed 103 grams and the largest 365 grams. It is seen that the spleen is only slightly enlarged and is not palpable. Pemberton[4] in a series of fifty-seven splenectomies for thrombocytopenic purpura found the average weight of the spleen to be 201.25 grams. However, he included in this series a spleen which weighed 700 grams in a 10-year-old child.

The histologic findings in the spleen are negligible. The majority of spleens had a fairly normal histologic structure. The Malpighian corpuscles are prominent as observed commonly in young people. The sinuses of the pulp are frequently filled and distended with small groups of red blood cells. Small collections of neutrophiles are fairly common. However, this is not sufficient to speak of a true splenitis. Slight fibrosis of the pulp was observed in one case. Nickerson and Sunderland[5] in five patients with essential thrombocytopenic purpura attached importance to the finding of megakaryocytes in the sinuses of the pulp. Megakaryocytes are occasionally seen in the sinuses of the pulp. The bone marrow in these patients usually contained large numbers of similar cells. Younger forms of megakaryocytes are difficult to distinguish from large reticulum cells of the splenic sinuses in the usual hematoxylin and eosin preparations. The lack of other signs of myeloid metaplasia in the spleen would indicate that these megakaryocytes are embolic from the bone marrow. Kaznelson[6] reported finding phagocytosis of the platelets by the reticulo-endothelial cells of the splenic sinuses. Others have failed to find this phenomenon. It is extremely difficult to study phagocytosis of platelets in paraffin sections. The study of smears from the splenic vein at time of operation and imprints of the spleen before fixation would be a distinct aid in this direction. One can conclude that the spleen shows no gross or microscopic changes which are characteristic of thrombocytopenic purpura.

In recent years, the value of bone marrow studies in essential thrombocytopenic purpura has been widely recognized. Post-mortem autolysis and embalming usually restrict bone marrow studies at autopsy to paraffin sections of fixed and decalcified material. Hence, one is at a disadvantage in studying minute cellular detail as compared to fresh material in the living patient stained with some modification of the Romanowsky stain. Unfortunately in these thirty-six patients, the bone marrow was examined in only ten. Sections

were available for study in eight of the ten. In the patients not available for study, the bone marrow was described as negative for leukemia and normal megakaryocytes. In the remaining eight, three died following splenectomy. These three patients showed a marked reduction in the number of megakaryocytes in the marrow. We are rapidly beginning to realize that in any patient with thrombocytopenic purpura, where the marrow shows a marked decrease in number of megakaryocytes, the patient is likely to receive little benefit from a splenectomy. This is well illustrated by the following case in this series.

A woman, 22 years of age, gave a history of bleeding from gums, vagina, and skin purpura beginning in February of 1942. Red blood count on admission to the hospital was 1,260,000 cells per cubic millimeter; hemoglobin, 33 per cent (Sahli); leukocyte count, 7,700 cells per cubic millimeter with 69 per cent neutrophiles; platelet count, 55,000 per cubic millimeter; bleeding time, over 16 minutes; and coagulation time, 4 minutes. Three bone marrow aspirations showed an absence of megakaryocytes and no evidence of leukemia. The patient was given six blood transfusions. On Feb. 28, 1942, a splenectomy was done as a possible lifesaving procedure. The platelet count rose but declined on the second postoperative day with a return of purpuric bleeding. The patient died on the fifth postoperative day from generalized purpura. The bone marrow at autopsy showed an absence of megakaryocytes.

The bone marrow in the five patients not operated upon showed a marked increase in the number of megakaryocytes with the presence of many immature megakaryocytes. Every high power microscopic field had from one to several large megakaryocytes. The degree of platelet production on the part of megakaryocytes could not be determined in the fixed tissue. These marrows otherwise appeared relatively normal except for a compensatory normoblastic erythroid hyperplasia and myeloid hyperplasia compatible with a chronic bleeding state. Eosinophilia was not noted. The question arises as to whether one should restrict the definition of essential thrombocytopenic purpura to only those cases that show a marked increase in number of megakaryocytes in the bone marrow together with the presence of immature megakaryocytes. However, some investigators, as Rosenthal,[7] refer to one type of idiopathic thrombocytopenic purpura where megakaryocytes are reduced in number. One would expect poor results from splenectomies in these cases.

We are still ignorant as to the nature of the capillary defect and relationship of the spleen, platelets, and capillaries in this disease. The large numbers of megakaryocytes in the bone marrow would tend to show, as emphasized by Limarzi and Schleicher[8] and Dameshek and Miller,[9] that the lack of platelets is the result of a splenic depressing factor that interferes with the proper maturation of the megakaryocytes in the bone marrow.

CONCLUSIONS

1. The post-mortem findings of thirty-six patients with essential thrombocytopenic purpura are recorded.

2. The average weight of the spleen in twenty adults was 232 grams. There were no characteristic histologic findings.

3. Apart from the widespread hemorrhages, the presence of increased number of megakaryocytes in the bone marrow is the most constant post-mortem finding in essential thrombocytopenic purpura.

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EXPERIENCE WITH PTEROYLGLUTAMIC (SYNTHETIC FOLIC) ACID IN THE TREATMENT OF PERNICIOUS ANEMIA

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THE synthesis of a compound known to have a specific stimulating effect on hematopoiesis in man and in certain laboratory animals was announced in August, 1945, by a group of sixteen investigators.[1] This substance, synthetic folic acid, was found to be identical with the *Lactobacillus casei* factor previously derived in pure form from liver.[2] Extracts containing a substance or substances essential to the growth of *Lactobacillus casei* (and *Streptococcus lactis R*) have been obtained from sources[3-6] other than liver, namely, kidney, yeast, and the green leaves of many plants, notably spinach. As yet it is not known, however, whether the active principle in these extracts is identical with the synthetic compound or with the factor derived from liver.* Spies and associates[7] considered synthetic folic acid to be a part of the vitamin B complex. The formula and the method of synthesis of the hepatic *L. casei* factor were published in May, 1946; "pteroylglutamic acid" was suggested as a name for this compound.[8]

In recent months reports have been published on the therapeutic effectiveness of pteroylglutamic (synthetic folic) acid in certain macrocytic anemias. The rationale of use of this substance in the treatment of anemias of the macrocytic type was based on the experimental work of Wills and associates[9, 10] in England and of Day and co-workers[11-14] in this country. These workers produced a dietary deficiency in monkeys characterized by macrocytic anemia, leukopenia, diarrhea, and oral lesions (necrosis and ulceration of the gums), a syndrome not unlike that of sprue in man. Day and co-workers postulated that the deficiency was due to an unknown substance for which the term "vitamin M" was proposed. Subsequently, the same group of investigators demonstrated that deficiency of vitamin M in monkeys could be treated successfully with a highly purified *L. casei* factor.[14, 15]

The similarities of the sprue syndrome in man to the manifestations of deficiency of vitamin M in monkeys led two groups of investigators, Spies and associates and Darby and co-workers, to study the effect of the synthetic compound in patients with sprue.[7, 16-22] Clinical improvement characterized by disappearance of the glossitis, regeneration of lingual papillae, subsidence of diarrhea, and gain in weight was noted. Concomitantly, a marked hematologic response manifested by reticulocytosis and a rise in values for hemoglobin and erythrocytes was observed.

Studies on the therapeutic value of pteroylglutamic acid in other types of macrocytic anemia—addisonian pernicious anemia, pernicious anemia of pregnancy, nutritional macrocytic anemia, and macrocytic anemia of infancy—now have been reported also.[7, 19, 23-25] Vilter and associates[16] and Moore and co-

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Read before the Minnesota Society of Internal Medicine, Oct. 21, 1946.

The pteroylglutamic (synthetic folic) acid employed in this study was furnished by Dr. Stanton M. Hardy, of the Lederle Laboratories, Inc., Pearl River, N. Y.

*Reference to vitamin B_c and allied substances has been omitted, since evidence indicates that, although related, they probably are not identical with pteroylglutamic acid. The physiologic action of vitamin B_c on hematopoiesis, however, appears to be similar to that of pteroylglutamic acid.

workers[23] reported simultaneously in December, 1945, on the effect of pteroylglutamic acid in addisonian pernicious anemia in relapse. To the results observed in the seven cases reported by these investigators, Doan and associates[24, 26] have added their observations in four cases, Kaufmann and Schwager[27] in two cases, Amill and Wright[28] in six cases, and Goldsmith[29] in four cases, making a total of twenty-three cases of pernicious anemia in relapse on which published data concerning the effects of pteroylglutamic acid therapy are available. In these twenty-three cases the administration of pteroylglutamic acid, orally or parenterally, resulted in a reticulocyte response, an increase in values for hemoglobin and erythrocytes, and clinical improvement. Although studies on the effects of this form of therapy on the bone marrow of patients who had pernicious anemia in relapse have been made in several of the reported cases, detailed studies are available in only two instances.[24, 26] In these cases the megaloblastic marrow of pernicious anemia in relapse was converted to a marrow which approaches normal from the tenth to the fourteenth day of treatment.

It is the purpose of this paper to record our observations on the effects of pteroylglutamic acid therapy in fourteen patients with classical addisonian pernicious anemia in relapse. We wish to emphasize particularly the changes observed in the bone marrow after the administration of this substance and the failure of the synthetic compound to relieve or prevent the development of gastrointestinal and neurologic manifestations of the disease.

MATERIAL AND METHODS

In nine of the fourteen cases included in this study the condition in question was seen for the first time, the diagnosis having been made just prior to treatment with pteroylglutamic acid. In the remaining five cases (Patients 1, 2, 3, 7, and 14) a sensitivity to liver extracts previously administered parenterally had developed in two (Patients 7 and 14), and the patients were in severe relapse at the time of their admission to the Mayo Clinic. Two others (Patients 2 and 3) in this group of five had been informed one year and three years previously that they had pernicious anemia, but they had neglected liver therapy over periods of many months. The fifth (Patient 1) had received a single intramuscular injection of liver extract one week before admission because his physician told him that he was anemic, although the diagnosis of pernicious anemia apparently had not been established. Eight of the fourteen patients were women and six were men. Their ages ranged from 48 to 72 years. Six of the fourteen patients were hospitalized during the first four to six weeks of treatment and were given a routine hospital diet. The remaining eight patients were seen daily or every second day in the clinic and were maintained on diets of an average protein content (70 Gm.).

During the first two weeks of treatment erythrocyte and reticulocyte counts were made daily; subsequently, such counts were made every second or third day. Reticulocyte percentages were obtained by counting 1,000 cells on dry films stained with brilliant cresyl blue and counterstained with Wright's stain (Grübler). The Sheard-Sanford photelometer was used for the determination of values for hemoglobin, and a Haden erythrocytometer was employed for the determination of average erythrocyte diameters. Sternal puncture was performed in twelve of the fourteen cases prior to the institution of therapy and was repeated at intervals in each case, so that in the aggregate at least one puncture was performed for each day from the second to the tenth day of treatment. Both the oral route and the parenteral route of administration of pteroylglutamic acid were employed.

RESULTS

Hematologic Response.—The quantity of pteroylglutamic acid administered, the route of administration, and the hematologic response in respect to

each of the fourteen patients is shown in Table I. Peaks of the increase in reticulocytes occurred from the fifth to the fourteenth day after the institution of therapy, and the increases varied from 1.6 to 17.6 per cent. These values, in general, are somewhat lower than those reported by other observers and are less than the reticulocyte peaks observed after liver therapy among patients who had pernicious anemia in similar states of relapse.

Although treatment with pteroylglutamic acid resulted in an increase in values for erythrocytes and hemoglobin in all cases, the rapidity of the increase was variable. In some cases the response appeared to be as rapid as that usually observed with liver therapy (Fig. 1), but in other cases it was decidedly delayed (Fig. 2). In three patients the values for erythrocytes did not increase above 3,500,000 cells per cubic millimeter of blood, despite daily doses of 15 to 20 mg. over periods as long as eleven weeks (Patients 2, 9, and 13). In these three patients normal blood values subsequently were induced with liver therapy. Recession in the degree of macrocytosis occurred in all cases, but a return to essentially normal cell diameters was observed in only three instances (Patients 4, 11, and 14).

Leukocyte counts of less than 5,000 cells per cubic millimeter were observed in six of the fourteen patients prior to treatment with pteroylglutamic acid. Treatment resulted in an increase in the number of leukocytes to normal in two cases (Patients 1 and 7), an increase with persistence of a subnormal value in two (Patients 8 and 14), and a decrease in two (Patients 2 and 12). Platelet determinations made prior to treatment with pteroylglutamic acid revealed thrombocytopenia in four of seven patients. After treatment, an increase in platelet values occurred in all four patients.

In the twelve patients for whom sternal puncture was performed megaloblastic regeneration in the bone marrow was found prior to treatment with folic acid. Treatment resulted in a rapid conversion to a normoblastic marrow, the conversion being essentially complete in seven to ten days. The changes observed are illustrated in material aspirated serially in a typical instance (Figs. 3 to 5).

Clinical Observations.—Although evaluation of subjective symptoms is more difficult than analysis of objective data, the impression was gained that in pernicious anemia in relapse treatment with pteroylglutamic acid resulted in less pronounced and less rapid subjective improvement than that commonly observed after the institution of liver therapy. With the exception of two cases in which the hematologic response to pteroylglutamic acid therapy was rapid, weakness persisted longer and the restoration of a sense of well-being, which often occurs with startling rapidity after the administration of liver extract, either was significantly delayed or did not occur.

The results of treatment with pteroylglutamic acid over periods of many months on the glossitis and neural manifestations of pernicious anemia are summarized in Table II. Of seven patients who had glossitis, six improved under treatment; however, the improvement of four of these was slower than that observed with liver therapy, since one to three and a half months of therapy with acid were required for restoration of a normal tongue. Three of the six patients experienced recurrence of glossitis at a later date; in two of them (Patients 2 and 7) satisfactory hematologic remission was not induced, but in

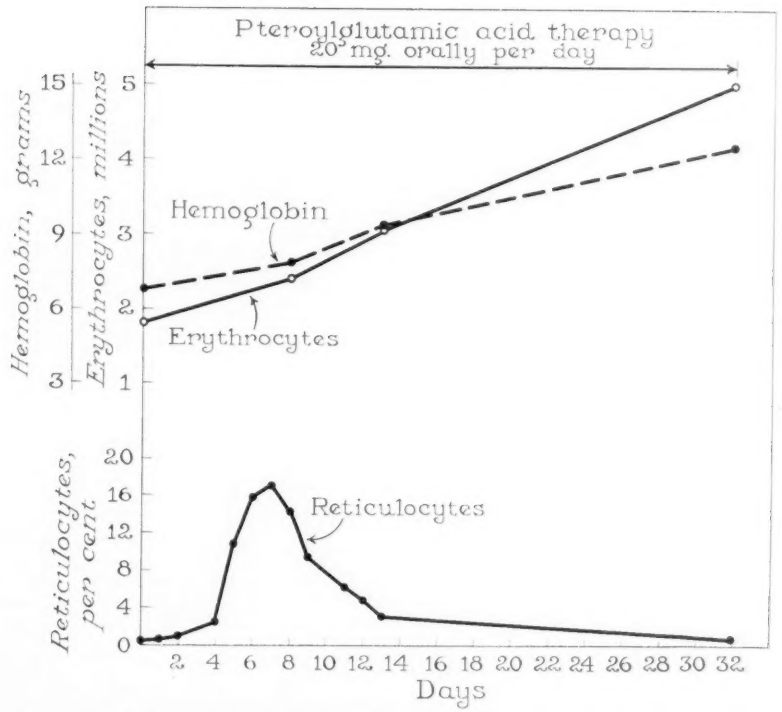

Fig. 1.—A satisfactory erythrocytic response (Patient 14) to pteroylglutamic acid therapy in pernicious anemia in relapse. The increase in values for erythrocytes and hemoglobin was rapid, and the reticulocyte response appeared to be either maximal or very nearly maximal.

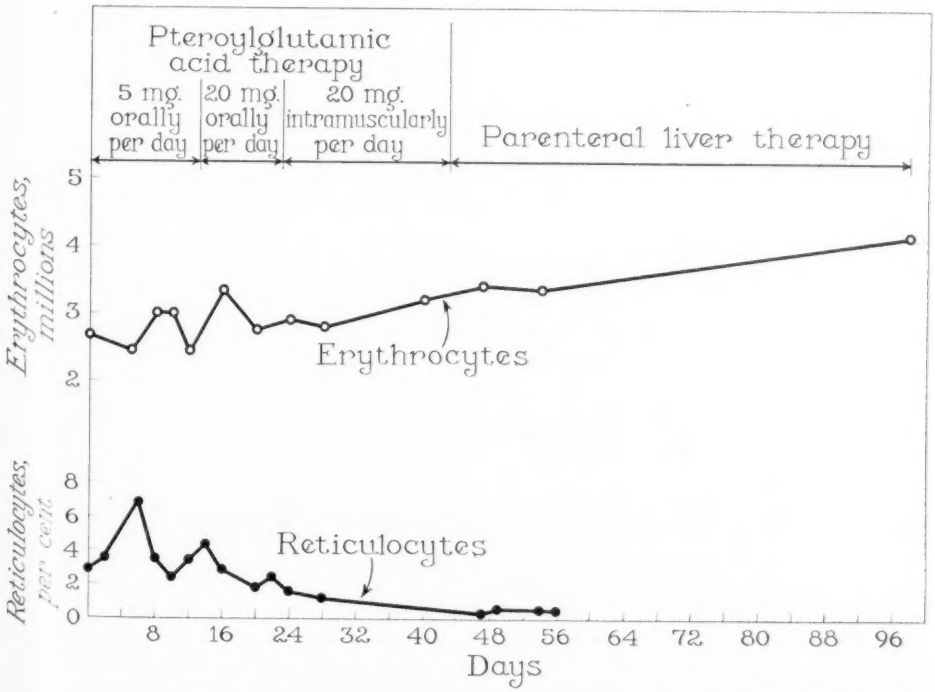

Fig. 2.—An unsatisfactory erythrocytic response (Patient 13) to pteroylglutamic acid therapy in pernicious anemia in relapse. Only a slight increase in the number of erythrocytes occurred over a period of forty-four days. The initial submaximal reticulocyte response may have been caused by the small dose administered during the first eleven days of treatment. The administration of larger doses both orally and parenterally did not result in a significant increase in the rate of regeneration of erythrocytes. Therapy with liver extract administered parenterally subsequently brought about restitution of normal blood values.

TABLE I. DOSAGE, ROUTE OF ADMINISTRATION, AND HEMATOLOGIC RESPONSE TO PTEROYLGLUTAMIC ACID THERAPY IN PERNICIOUS ANEMIA

PATIENT	AGE	SEX	PTEROYLGLUTAMIC ACID THERAPY			HEMOGLOBIN (GRAMS)		ERYTHROCYTES (MILLIONS)				RETICULOCYTES			ERYTHROCYTOMETER READING (MICRONS)		LEUKOCYTES (THOUSANDS)		PLATELETS (THOUSANDS)	
			ADMINISTRATION (ROUTE)	DAILY DOSE (MG.)	DURATION OF TREATMENT (DAYS)	INITIAL	FINAL	INITIAL	FOURTEENTH DAY	THIRTIETH DAY	FINAL	FIRST DAY OF RISE	DAY OF PEAK	PER CENT AT PEAK	INITIAL	FINAL	INITIAL	FINAL	INITIAL	FINAL
1	65	M	Oral	100	1 to 37	10.1	13.1	2.44	3.32	3.96	4.19	8	14	6.4	8.3	7.8	4.8	6.6		202
2	72	M	Oral I.M. Oral	100 20 15	1 to 14 15 to 36 37 to 79	6.2	10.3	1.61	2.36	3.44	3.43	3	5	10.2	8.6	7.5	4.4	2.7		119
3	66	M	Oral Oral Oral	50 15 10	1 to 14 15 to 71 72 to 147	12.0	11.9	3.21	3.51	(43)* 3.88	3 94	5	8	6.3	8.0	7.7	6.8	8 9	176	147
4	64	F	Oral Oral Oral Oral	10 5 10 20	1 to 33 34 to 99 100 to 165 166 to 213	10.0	13.1	2.72	3.74	(32) 4.27	4.58	3	6	8.9	8.0	7.1	8.1	8.3	185	119
5	66	M	Oral Oral Oral Oral	25 15 20 10	1 to 14 15 to 27 28 to 96 97 to 218	9.8	10.8	2.78	3.22	(26) 3.49	3.97	5	11	3.7	8.4	7.5	7.7	8.6		106
6	52	F	I.M. Oral Oral	20 10 5	1 to 21 22 to 55 56 to 261	5.3	13.8	1.28	3.25	(21) 4.15	4.22	6	12	4.5	8.3	7.8	6.5	11.5	58	165

*Numbers in parentheses denote day on which determination was made, if different from thirtieth day.

7	68	F	I.M.	10	1 to 20	4.0	11.2	1.15	2.43	(28) 3.20	3.21	3	5	17.6	8.3	7.7	3.4	6.5	67	130
			I.M.	20	21 to 30															
			Oral	15	31 to 160															
8	67	F	I.M.	20	1 to 14	3.7	11.7	1.00	2.76	3.47	4.52	4	9	13.8	8.1	7.7	2.0	4.7	80	116
			I.M.	10	15 to 26															
			Oral	10	27 to 41															
			Oral	25	42 to 46															
			Oral	15	47 to 55															
9	66	F	I.M.	20	1 to 24	10.5	11.6	3.05	3.23	3.38		7	8	4.5	8.3	8.0	4.0			
10	65	M	I.M.	20	1 to 42	11.2	11.9	2.57	3.24	3.99		3	7	4.5	8.3		7.3			
11	50	F	Oral	5	1 to 10	8.7	13.8	2.40	3.13	3.97	4.68		7	14.4	8.0	7.2	6.1	6.9		
			Oral	20	11 to 68															
			Oral	10	69 to 167															
12	60	F	I.M.	10	1 to 10	13.3	12.9	3.69	3.84	3.54	4.20	4	4	1.6		7.6	7.2	4.8		
			I.M.	20	11 to 25															
			Oral	20	26 to 70															
			Oral	15	71 to 136															
			Oral	30	137 to 193															
13	48	F	Oral	5	1 to 11	8.9		2.67	2.65	(28) 2.80	3.21	3	7	6.9	8.0	7.8	7.2	4.9	171	119
			Oral	20	12 to 23															
			I.M.	20	24 to 44															
14	55	M	Oral	20	1 to 32	6.5	14.0	1.44	3.06	(32) 5.01	4.82	5	8	16.7	8.0	7.2	3.9	4.5	55	166
			Oral	10	33 to 87															

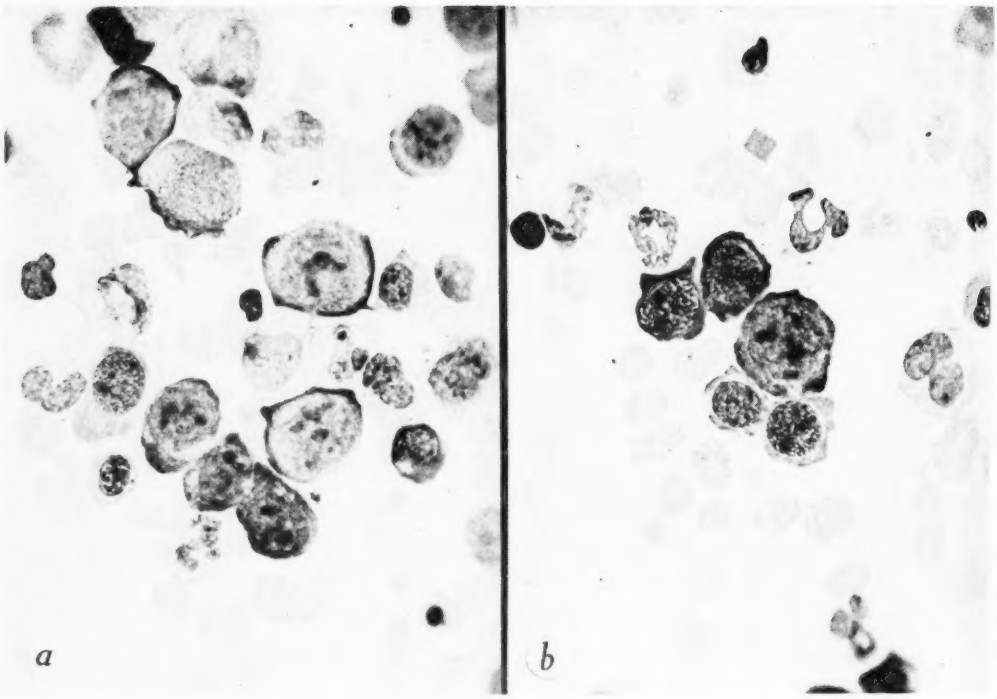

Fig. 3*a* and *b*.—Specimens of sternal bone marrow aspirated serially in a patient with addisonian pernicious anemia (Patient 2), during relapse before treatment with pteroylglutamic acid, depicting a predominance of promegaloblasts and basophilic megaloblasts (×650).

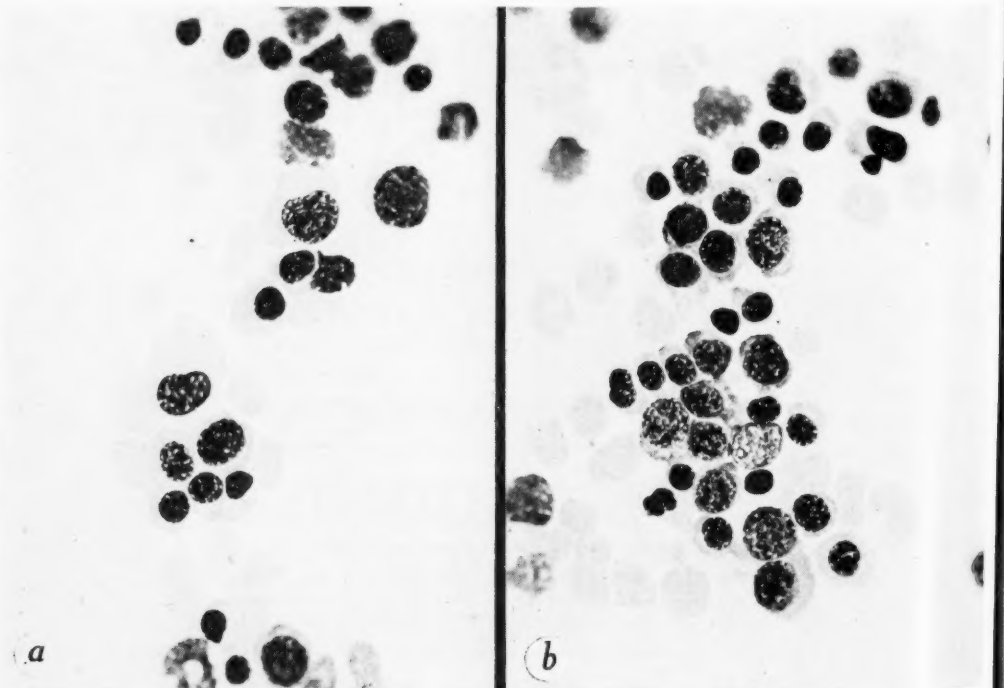

Fig. 4*a* and *b*.—*a*, Same case as concerned in Fig. 3. Specimen of sternal marrow three days after the institution of pteroylglutamic acid therapy, showing late megaloblastic maturation, manifested by a predominance of orthochromatic megaloblasts (×650); *b*, normoblastic regeneration in same specimen as shown in Fig. 4*a*, as evidenced by basophilic, polychromatophilic, and orthochromatic normoblasts (×650).

the third (Patient 8) the blood picture was essentially normal at the time of recurrence of the glossitis except for persistence of a slight degree of macrocytosis. The one (Patient 9) who obtained no abatement of the glossitis was treated with pteroylglutamic acid for only twenty-four days. Although therapy apparently failed in this instance, the period of observation was too short to permit the formation of conclusions.

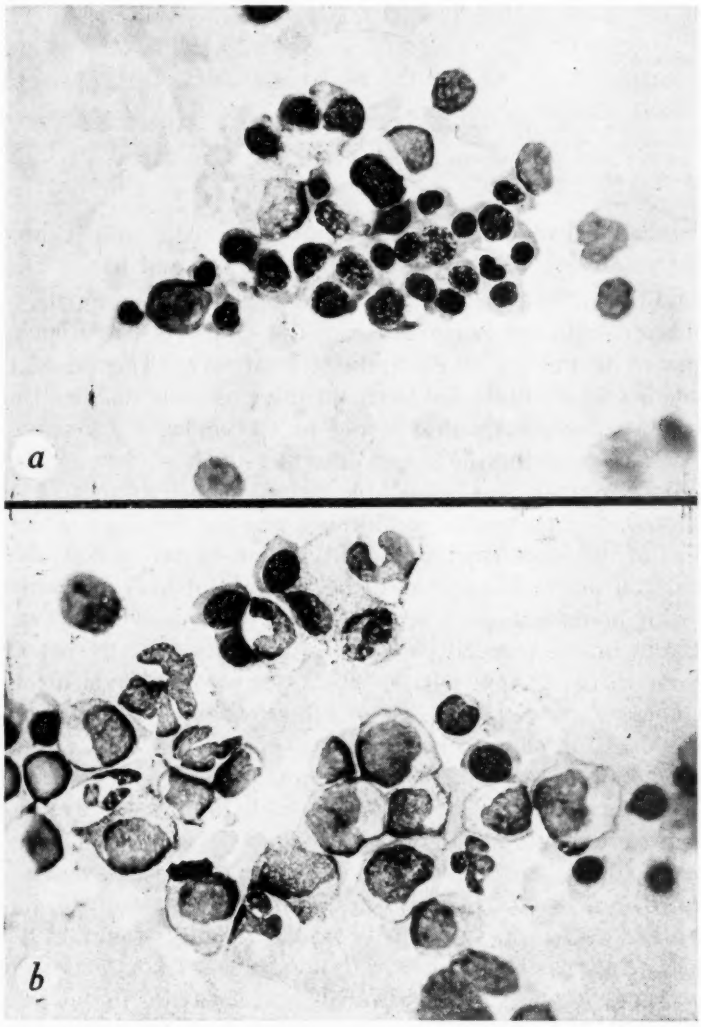

Fig. 5*a* and *b*.—*a*, Same case as concerned in Figs. 3 and 4, showing specimen of sternal bone marrow eight days after institution of pteroylglutamic acid therapy, depicting active normoblastic regeneration; a few scattered megaloblasts could still be found at this stage (×650); *b*, specimen of sternal marrow thirty-four days after the institution of pteroylglutamic acid therapy, showing resumption of active myelopoiesis as well as less intensive normoblastic regeneration (×650).

Of the ten patients who had paresthesia in the extremities, eight improved, but the improvement of four patients was temporary. It is of interest that paresthesia recurred within two to four months after the institution of therapy, at a time when three of the four patients no longer were anemic (Patients 4, 8, and 12). Of even greater interest is the observation that in two patients

TABLE II. EFFECT OF ADMINISTRATION OF PTEROYLGLUTAMIC ACID ON GLOSSITIS AND NEURAL MANIFESTATIONS IN PERNICIOUS ANEMIA*

SIGN OR SYMPTOM	INSTANCES OBSERVED				
	BEFORE TREATMENT	AFTER TREATMENT			
		IMPROVED, NO RECURRENCE	IMPROVED TEMPORARILY, RECURRENCE	UNIMPROVED OR WORSE	FIRST APPEARANCE DURING TREATMENT
Glossitis	7	3	3	1†	0
Paresthesia	10	4	4	2	2
Subacute combined degeneration of the spinal cord	2	0	0	2†	3
Total	19	7	7	5	

*Based on the study of fourteen patients.
†Short period of observation.

who had not had paresthesia prior to treatment, this symptom developed five months after treatment had been started (Patients 3 and 6).

The condition of two patients who had peripheral neuropathy and early signs of subacute combined degeneration of the spinal cord (Patients 1 and 10) did not improve during five or six weeks of treatment. The size of the dose of pteroylglutamic acid administered to one of these patients was five times greater (100 mg. daily) than that administered to the other, but during the short period the patient receiving the larger dose was under observation before treatment with liver extract was begun, the neural manifestations of the disease were not altered. Of particular significance was the development of early but definite signs of subacute combined degeneration of the spinal cord in three patients who had not had involvement of the central nervous system prior to treatment with pteroylglutamic acid (Patients 3, 7, and 8). Two, however, had paresthesia in the extremities which abated temporarily after treatment and then recurred two to four months later. Signs of involvement of the spinal cord in these cases were loss of sense of vibration and sense of position in the lower limbs, slight incoordination, presence of the Romberg sign, slight but definite ataxia, and, in one patient, the bilateral presence of the Babinski great toe sign. The neural manifestations developed within two to five months after treatment with pteroylglutamic acid had begun. In one case (Patient 8) they developed at a time when the number of erythrocytes was normal, although a slight degree of macrocytosis persisted. In another (Patient 3), in which the patient who had not had symptoms or signs of involvement of the nervous system prior to treatment with pteroylglutamic acid, a satisfactory hematologic remission was not induced, as manifested by persistence of macrocytosis and a value for erythrocytes that did not increase above 4,000,000 cells per cubic millimeter of blood, except on one occasion. One case (Patient 7) is of special interest because the erythrocyte count increased from 1,150,500 to 4,000,000 cells per cubic millimeter over a period of four months and then decreased to 3,210,000 cells per cubic millimeter in the next five weeks (Fig. 6). During the period of decrease in number of erythrocytes and with the patient receiving a daily maintenance dose of 15 mg. of pteroylglutamic acid by mouth, glossitis and paresthesia in the extremities recurred and signs of subacute combined degeneration of the spinal cord developed. Inquiry into the diets of three patients revealed essentially normal diets in two (Patients 3 and 8), but the

diet in one (Patient 7) was low in protein. In the latter instance the patient estimated that she had not received in excess of a half pound of animal protein weekly for a period of two months prior to the development of glossitis and the symptoms of involvement of the nervous system. She attributed the low intake of protein to the meat shortage prevailing during the summer of 1946.

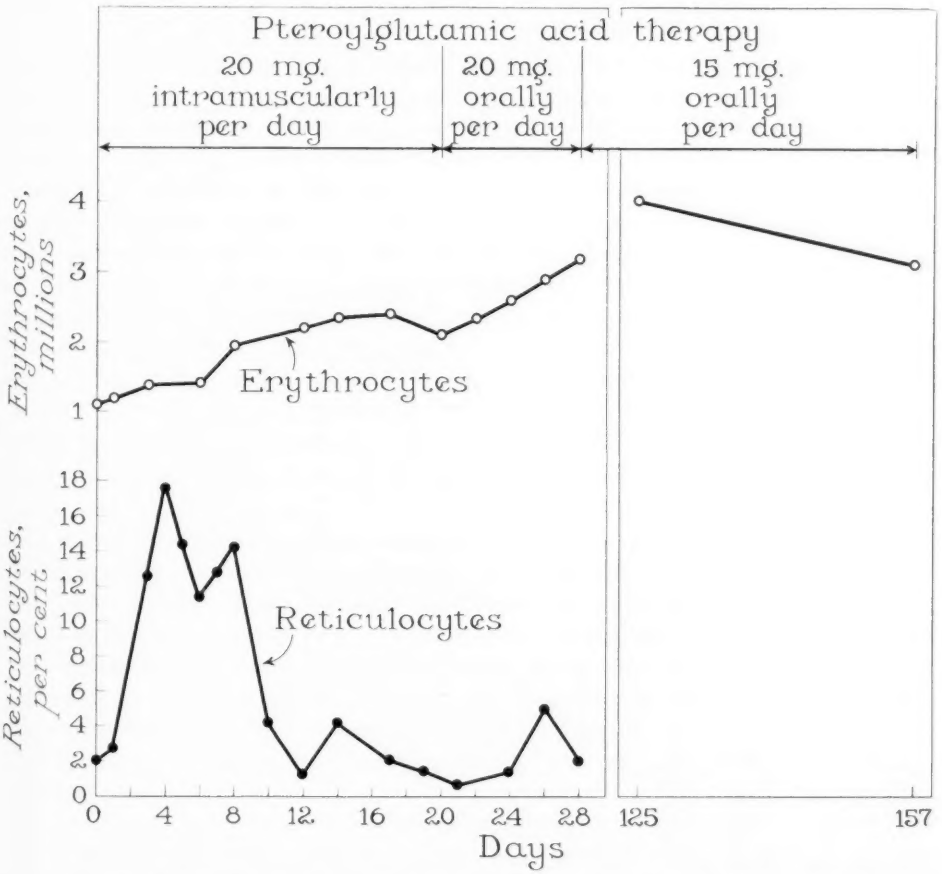

Fig. 6.—Diminution of the number of erythrocytes per cubic millimeter of blood in a patient with pernicious anemia receiving orally a daily dose of 15 mg. of pteroylglutamic acid, after an apparently satisfactory initial response to such therapy. In this case (Patient 7) relapse was due to cessation of liver therapy, many months prior to the patient's admission, because of sensitivity to liver extracts. The presenting symptoms were glossitis and paresthesia in the lower extremities, but the signs of combined systemic disease were absent. During the first two months of treatment the glossitis and paresthesia abated but then recurred and became moderately severe during the last few weeks of treatment. During the period of the decrease in erythrocytes, the early signs of subacute combined degeneration of the spinal cord developed. Desensitization of the patient to liver extract administered parenterally then was carried out, and intensive liver therapy subsequently resulted in restitution of normal blood values and symptomatic improvement.

COMMENT

Pteroylglutamic acid appears to be an essential nutrient for man, for various laboratory animals, and for certain microorganisms. An important physiologic property is the stimulating effect of the acid on hematopoiesis, which is most pronounced in certain, but not all, types of macrocytic anemia in man characterized by megaloblastic regeneration in the bone marrow. In this type of anemia, including addisonian pernicious anemia, the administration of the

synthetic compound results in the prompt conversion of a pathologic megaloblastic form of erythropoiesis to a normal normoblastic form of erythropoiesis. Concomitant with the changes observed in the bone marrow, a hematologic response occurs, characterized by reticulocytosis and an increase in values for hemoglobin and erythrocytes. An increase in the number of leukocytes and platelets also occurs in most cases in which leukopenia and thrombocytopenia are found prior to treatment.

Although the foregoing remarks on the hematopoietic-stimulating effect of pteroylglutamic acid applied, in general, to all cases of patients with pernicious anemia in our series, considerable variation in the rate of hematologic improvement occurred. Only three of fourteen patients who had pernicious anemia in relapse treated with pteroylglutamic acid showed an erythrocyte response comparable to that which occurs after liver therapy. In the remaining eleven cases the rate of increase of erythrocytes was significantly slower than the expected response to treatment with liver extract. In four cases normal values for erythrocytes were not attained after eight to ten weeks of treatment. Subsequent intensive liver therapy, however, resulted in the restitution of normal blood values in these four cases. It is of interest to note also that in the majority of our cases the reticulocyte responses to treatment with pteroylglutamic acid were lower than the standard maximal reticulocyte responses that have been established for various erythrocyte levels after the intramuscular administration of liver extract.[30-32]

Clinical improvement after pteroylglutamic acid therapy, as well as the hematologic response, was found to be variable in patients who had pernicious anemia in relapse, and often it was slower than the response observed with liver therapy. In a significant proportion of cases glossitis and neurologic manifestations of the disease either failed to abate or recurred after temporary abatement. Of greater significance was the development of signs of subacute combined degeneration of the spinal cord in three of the fourteen patients treated with pteroylglutamic acid in doses of 10.0 to 15.0 mg. administered daily by mouth for periods of two to five months.* These observations suggest that the physiologic action of pteroylglutamic acid on the alimentary tract and nervous system in pernicious anemia is not identical with that of the antipernicious anemia factor contained in liver, even though the action on the bone marrow is similar.

Although pteroylglutamic acid has antianemic properties, it is probable that this factor and the antipernicious anemia factor contained in liver are not the same. The quantity of pteroylglutamic acid contained in liver extracts is small. Clark[33] reported that various commercial liver extracts contain 0.25 to 0.5 micrograms of the *L. casei* factor per U.S.P. unit. Thus, a patient receiving 150 units of the antipernicious anemia factor in liver extract (10 c.c.) during the first ten days of treatment would receive only 5.5 micrograms of pteroylglutamic acid. Doses required for hemopoietic response are considerably larger. In our study the minimal amount of pteroylglutamic acid administered

*Since this paper was written, Vilter, Vilter, and Spies, at the annual meeting of the Central Society of Clinical Research, November, 1946, reported the development of combined systemic disease in four persons with pernicious anemia after five to eight months of treatment with pteroylglutamic acid. The administration of 50 to 500 mg. of the *L. casei* factor for ten to forty days did not result in subjective or objective improvement. We have learned also, in a personal communication, that L. Meyer of New York City previously had reported similar observations.

to the patient during the first ten days of treatment was 50,000 micrograms. However, hemopoietic responses have been obtained with as little as 20,000 micrograms during the first ten days of therapy.[24]

It is obvious that the discovery and synthesis of pteroylglutamic acid are important advances in the fields of nutrition and hematology. The observation that this substance does not prevent the development of glossitis and neural manifestations in pernicious anemia, even after the induction of hematologic remission, in no way detracts from the significance of the demonstration that deficiency of a substance of known chemical composition profoundly inhibits erythrocytic maturation, and possibly the maturation of other elements, in the bone marrow. Although present evidence indicates that pteroylglutamic acid should not be employed as a form of replacement or substitution therapy in pernicious anemia, whether in relapse or remission, data concerning the usefulness of this agent in the treatment of other types of macrocytic anemia related to pernicious anemia are accumulating rapidly. In our experience pteroylglutamic acid therapy has been found to be particularly effective in the treatment of nutritional macrocytic anemia and macrocytic anemia of infancy.

SUMMARY AND CONCLUSIONS

Fourteen patients who had addisonian pernicious anemia in relapse were treated with pteroylglutamic (synthetic folic) acid over periods from twenty-four days to nine months. The most striking effect of this form of therapy was observed in the bone marrow, erythropoiesis rapidly changing from a megaloblastic to a normoblastic type. However, considerable variation in the rate of erythrocytic regeneration was encountered, and in certain instances normal blood values were not obtained after several months of treatment with pteroylglutamic acid in doses generally thought to be relatively large.

Symptomatic improvement also was variable. Treatment resulted in abatement of glossitis and peripheral neuropathy in most cases, but recurrence was common among persons maintained solely on this form of therapy for a period of months. Moreover, peripheral neuropathy and subacute combined degeneration of the spinal cord developed as new manifestations in a significant proportion of cases two to five months after treatment was begun. In the light of these observations it is obvious that pteroylglutamic acid does not prevent the occurrence of degenerative disease of the peripheral nerves and spinal cord in pernicious anemia and that the use of this form of therapy as a substitute for extracts of liver or gastric mucosa subjects patients to the hazards of progression or development of the neural manifestations of the disease. On the other hand, pteroylglutamic acid has been found to be an effective therapeutic agent in certain macrocytic anemias related to pernicious anemia, a subject not considered in the present paper.

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THE EFFECT OF SYNTHETIC LACTOBACILLUS CASEI FACTOR ON THE BLOOD CHANGES INDUCED BY GASTRECTOMY IN THE RAT

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ONE of the newest members of the group of water-soluble B vitamins is folic acid (synthetic *Lactobacillus casei* factor). The recognition of its function in the animal organism and its final synthesis[1] were the result of widely independent investigations in several laboratories extending over a period of fifteeen years or more. Factors found essential for the growth of microorganisms (*L. casei* and *Streptococcus lactis R*) and factors found essential for the nutrition of the chick (B_c) and of the monkey (vitamin M) may prove to have common physiologic significance.

Snell and Peterson[2] in 1940 found that a yeast extract and a solubilized liver fraction were good sources of a growth factor essential for *L. casei*. Mitchell and associates[3] in 1941 first used the term "folic acid" to describe a factor which they obtained from spinach and which is essential for the growth of *Str. lactis R*. Liver, yeast, and other substances proved to be rich sources of this factor. Hogan and Parrott[4] in 1939 used the term "vitamin B_c" to designate a dietary factor, obtained from a water-soluble extract of liver, which prevented macrocytic hyperchromic anemia in chicks. Pfiffner and co-workers[5] in 1943 isolated a crystalline compound from liver, and Campbell and associates[6] in 1944 showed that this compound would prevent nutritional macrocytic anemia in chicks. The designation of Hogan and Parrott (vitamin B_c) was retained. The term "vitamin M" was employed by Day and associates[7] in 1938 to designate that factor in yeast and in liver which protected monkeys from fatal dietary cytopenia. In 1945 Day and co-workers[8] expressed the belief that *L. casei* factor is identical with vitamin M.

On the recognition that substances so widely scattered in nature are essential growth factors and possess antianemic potencies, numerous reports of the influence of liver and yeast concentrates, known to contain *L. casei* factor, were reported. Experimental anemia was induced in various ways. Spicer and associates[9] in 1942 reported that the leukopenia induced in rats by sulfonamide drugs could be prevented with liver extracts containing this factor. Kornberg and associates[10] in 1943 were able to correct severe granulocytopenia. By means of a folic acid concentrate Ransone and Elvehjem[11] in 1943 increased the leukocyte production in rats given sulfasuxidine. Likewise, Axelrod and co-workers[12] in 1943 corrected experimental leukopenia with a norite eluate factor or with whole liver. Higgins[13] in 1944 reported that a vitamin B_c concentrate had a marked antianemic effect on rats given promin.

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After the isolation of vitamin B_c by Pfiffner and associates[5] in 1943 and of the *L. casei* factor by Hutchings and co-workers[14] in 1944, additional studies using purified products were reported. Using crystalline folic acid, Daft and Sebrell[15] in 1943 reported the correction of experimental granulocytopenia. Mallory and associates[16] in 1944 increased granulocyte percentages in rats made leukopenic with succinylsulfathiazole. Spontaneous granulocytopenia observed in a small percentage of rats fed purified diets was corrected by the *L. casei* factor (Kornberg and co-workers[17] in 1945). Likewise, *L. casei* factor was found by Kornberg and co-workers[18] in 1944 to have a preventive as well as corrective action on induced hemorrhagic anemia. Granulocytopenia induced by thiourea, when thyroxin or powdered thyroid was given simultaneously, was found by Daft and associates[19] in 1946 to be corrected by crystalline *L. casei* factor. But neutropenia induced by the injection of benzol, a bone marrow depressant, was not corrected by folic acid, according to Platt and co-workers[20] in 1946. The neutropenia following administration of sulfonamide compounds was considered to be due to a decrease of the number of coliform organisms which presumably synthesize the *L. casei* factor in the lower part of the intestinal tract. Exogenous folic acid thus was able to correct the induced neutropenia.

The extensive literature covering the reports of studies on the effects of *L. casei* factor on various cases of clinical anemia was reviewed by Berry and Spies[21] in 1946. Synthetic *L. casei* factor appeared to exert striking antianemic effects on the several types of macrocytic anemia and was found effective in inducing remissions in some cases of pernicious anemia. Goldsmith,[22] reporting late in 1946 her study of five patients who had severe anemia, stated that there was improvement in their physical condition as well as in their hematologic status on the administration of *L. casei* factor. Bethell and collaborators[23] in 1946 stated that therapeutic effects were not obtained when the B_c conjugate was given to a gastrectomized patient, but an equivalent amount of folic acid incited a significant hematologic response.

Experimental gastrectomy has been shown to induce marked hematologic changes in some species of animals. The literature is extensive, and space does not permit a comprehensive review of the available reports. Briefly, the total removal of the stomach from rats (Jung,[24] 1933; Maison and associates,[25] 1933; Bussabarger and Jung,[26] 1936; and Ivy,[27] 1940) induced changes of body weight and severe hematologic changes including marked microcytosis. Large amounts of ferric ammonium citrate did not alter the weight curve but did improve the blood picture strikingly. A complete return to normal levels was difficult to attain with iron alone, but the administration of a concentrate of a liver protein with iron was reported to correct the hematologic dyserasia. It was obvious that the stomach is an essential organ for the maintenance of health in the rat, but blood findings in any way resembling those of pernicious anemia did not ensue on its removal.

Since synthetic *L. casei* factor has been shown to be corrective for certain clinical anemias, and since it has been shown to be effective in controlling cer-

tain nutritional anemias and other experimental anemias thought to be induced by altering intestinal flora by various drugs, a study was made of its effectiveness in correcting the blood changes incident to the removal of the stomach.

EXPERIMENTAL PROCEDURES

Healthy adult male rats of the Sprague-Dawley strain were selected. Gastrectomy was performed with the rats under ether anesthesia, the esophagus being joined to the duodenum by an end-to-side anastomosis. Sixteen animals were gastrectomized; eight were not submitted to operation and served as the control group. All animals, both control and test, were provided a purified diet consisting of 64 per cent sucrose, 24 per cent vitamin-free casein (Labco brand), 6 per cent corn oil, 2 per cent cod-liver oil, and a 4 per cent salt mixture No. 2, U.S.P. XII. Adequate amounts of thiamine, riboflavin, pyridoxine, niacin, calcium pantothenate, choline, inositol, and para-amino-benzoic acid were supplied. Water was provided ad libitum. All animals were maintained in metal cages, on metal screen three mesh to an inch, three animals to a cage. The average food intake was recorded daily for three two-week periods: immediately after operation, two months after operation, and seven months after operation. Animals were weighed at intervals of two weeks.

Samples of blood, always obtained from the hearts of both control and test animals, were examined at three weeks, six weeks, three months, and five months after gastrectomy. Shortly after the five-month sampling, 200 micrograms of synthetic *L. casei* factor* were given to each control and test animal by stomach tube. Seventy-two hours later the blood was again examined and data were assembled.

Two weeks later when the effects of the previous administration were presumably gone, the animals were fed the purified diet to which *L. casei* factor was added at a level of 100 micrograms per gram. After fourteen days on this dietary regimen the blood was again sampled and the data were assembled.

The purified diet, without the *L. casei* supplement, was again provided to the animals. After an interval of time sufficient to free the animals of the orally administered vitamin, all gastrectomized rats were given, each day for fourteen days, 400 micrograms of *L. casei* factor, intraperitoneally. At the end of this time the blood was again sampled and corresponding data were assembled.

RESULTS

All animals withstood the immediate postoperative period reasonably well. Three died of obstruction at the site of the anastomosis within the first two weeks. Three died as a result of hemopericardium occasioned by heart puncture, and four died during the summer for reasons presumably unrelated to the study. In cases of obstruction an abscess usually developed at the site of anastomosis, and the body weight of the animal dropped rapidly. Only the six gastrectomized animals which survived the initial five-month period and were without obvious pathologic defects were used in the computation of the following data.

A. Food Intake.—In the initial period, soon after gastrectomy, control animals ate on the average 15.1 Gm. (63.4 calories) of the diet per day, while the gastrectomized animals took 12.7 Gm. (52.3 calories) per day. Two months later, the nongastrectomized control animals were eating 10.7 Gm. (44.9 calories), while the gastrectomized animals were eating approximately an equal amount, 10.3 Gm. (43.2 calories) per day. This period of measuring the food intake was in midsummer, and it is our belief that the decline in the amount

*The pteroylglutamic (synthetic folic) acid employed in this study was furnished by Dr. Stanton M. Hardy of the Lederle Laboratories, Inc., Pearl River, N. Y.

consumed by the controls may have been owing to the higher temperatures and to the increased humidity levels of the season. Seven months after the operation, the control animals were eating on the average 11.7 Gm. (49.1 calories), while the gastrectomized animals were taking 8.9 Gm. (37.4 calories) daily. Although the food intake appeared to be quite satisfactory, only slightly less being consumed by the gastrectomized animals than by their controls; yet, the gastrectomized animals appeared undernourished, their coats were rough and unkempt, and all showed marked evidence of nutritional deficiencies.

B. Body Weights.—The average weight of the eight nongastrectomized control animals at the beginning of the experiment was 320 grams; that of the sixteen gastrectomized animals, constituting the test group, was 310 grams. The body weight data of the test group given in Table I were obtained from the six gastrectomized animals which had survived for five months and which were continuously free of any obvious pathologic defect.

TABLE I. BODY WEIGHTS IN GRAMS

	CONTROL	TEST
Preoperative	320	310
Postoperative		
One week	337	296
Two weeks	352	305
One month	364	318
Two months	380	324
Five months	372	236
Seven months	335	207

It is obvious from these data that the gastrectomized animals lost weight for a brief period and then increased it slightly for two months after the operation. Their weight then fell rapidly, a third of their preoperative weight having been lost at seven months. This loss may be owing to the fact that their food intake at this period was also nearly a third less than the amounts consumed daily during the early period after gastrectomy.

C. Effect on the Blood of a Single Oral Administration of Synthetic L. Casei Factor.—Data assembled from the heart bloods of normal and gastrectomized rats, five months after operation, before, and seventy-two hours after giving 200 micrograms of *L. casei* factor by mouth are condensed in Tables II

TABLE II. BLOOD DATA FIVE MONTHS AFTER GASTRECTOMY

	ANIMALS	ERYTHROCYTES (MILLIONS PER C.MM.)	ERYTHROCYTES (VOLUME IN CUBIC MICRONS)	HEMOGLOBIN (GM. PER 100 C.C.)	LEUKOCYTES (THOUSANDS PER C.MM.)	RETICULOCYTES (PER CENT)
Before Giving L. casei Factor						
Control	4	7.96 ± 0.2*	53.4 ± 2.5	14.3 ± 0.5	11.8 ± 1.1	1.3 ± 0.1
Gastrectomized	6	8.96 ± 0.1	38.9 ± 1.3	9.0 ± 0.8	10.7 ± 1.5	4.1 ± 0.4
Seventy-Two Hours After Giving 200 Micrograms of Synthetic L. casei Factor Orally to Control and Gastrectomized Animals						
Control	4	8.60 ± 0.3	46.4 ± 2.2	13.7 ± 0.2	9.8 ± 0.7	3.6 ± 0.6
Gastrectomized	6	9.30 ± 0.2	35.5 ± 0.5	8.6 ± 0.7	10.8 ± 1.1	6.4 ± 0.7

*Probable error of the mean.

TABLE III. BLOOD DATA FIVE MONTHS AFTER GASTRECTOMY

	Differential distribution (per cent)							TOTAL NEUTROPHILS (THOUSANDS PER C.MM.)
			GRANULOCYTES					
					NEUTROPHILS			
	LYMPHOCYTES	MONOCYTES	EOSINOPHILS	BASOPHILS	MYELOCYTES	METAMYELOCYTES	MATURE	
Before Giving L. casei Factor								
Control	60.0 ± 3.9*	0.5	3.5 ± 0.5	0.4	3.7 ± 0.6	4.2 ± 0.9	27.7 ± 2.7	4.2 ± 0.5
Gastrectomized	47.6 ± 1.5	0.6	0.6	0	6.2 ± 0.5	2.8 ± 0.6	42.2 ± 2.2	5.6 ± 0.9
Seventy-Two Hours After Giving 200 Micrograms of Synthetic L. casei Factor Orally								
Control	53.6 ± 3.9	1.7	3.5 ± 0.5	0.2	9.0 ± 0.8	2.0 ± 0.4	30.0 ± 2.8	3.4 ± 0.1
Gastrectomized	44.3 ± 2.0	0.5	0.1	0	11.2 ± 0.7	3.6 ± 0.6	40.3 ± 2.5	5.8 ± 0.5

*Probable error of the mean.

and III. Healthy gastrectomized animals never had low total erythrocyte counts. The mean of the data obtained from thirteen animals three weeks after operation was 8,550,000 ± 200,000 cells per cubic millimeter of blood. At that time their erythrocyte volumes and their hemoglobin levels did not differ from those values found in the nongastrectomized control animals. At five months, however, there was significant microcytosis and the amount of hemoglobin in grams per 100 c.c. of blood was greatly reduced.

A single administration of *L. casei* factor was without significant effect on the total erythrocyte count, the erythrocyte volumes, the grams of hemoglobin, or the total leukocyte count. Slightly significant elevations, however, were obtained in the reticulocyte percentages. In the data on leukocytes we observed some increase of the percentages of myelocytes in the blood of both control and test animals, but the total number of neutrophilic leukocytes was not increased. The conclusion was indicated that a single oral administration of the factor, in the amounts given, was without significant effect on the blood, seventy-two hours later.

D. Effect on the Blood of Including Synthetic L. Casei Factor in the Diet, at a Level of 100 Micrograms Per Gram.—Data assembled before placing animals on the diet, supplemented by *L. casei* factor, and those assembled after feeding this diet for two weeks are condensed in Tables IV and V. Although control animals took an average of 1,160 micrograms of the factor daily and test animals took an average of 920 micrograms daily, significant changes were not observed in the total number of erythrocytes per cubic millimeter, the volumes of erythrocytes, or the hemoglobin levels in gastrectomized animals. Again, there was a significant increase in the reticulocyte percentages in the test series. Although the percentage of lymphocytes in the blood of test animals dropped from 61.6 to 30.7, the total number of lymphocytes remained unchanged as a result of taking large amounts of the factor for fourteen days.

A marked increase, however, occurred in the percentage and in the total number of immature and mature neutrophilic granulocytes. These increments

TABLE IV. BLOOD DATA

	ANIMALS	ERYTHROCYTES (MILLIONS PER C.MM.)	ERYTHROCYTES (VOLUME IN CUBIC MICRONS)	HEMOGLOBIN (GM. PER 100 C.C.)	LEUKOCYTES (THOUSANDS PER C.MM.)	RETICULOCYTES (PER CENT)
Prior to Giving Synthetic L. casei Factor						
Control	3	8.43 ± 0.3*	50.5 ± 0.2	11.4 ± 0.2	8.0 ± 0.6	2.3 ± 0.3
Gastrectomized	5	8.46 ± 0.2	36.9 ± 1.7	6.9 ± 0.3	5.2 ± 0.7	3.2 ± 0.3
Two Weeks Later: Synthetic L. casei Factor in Diet at Level of 100 Micrograms per Gram; Average Daily Intake: Controls, 1,160 Micrograms and Gastrectomized Animals, 920 Micrograms						
Control	3	8.53 ± 0.1	50.4 ± 3.8	15.1 ± 0.6	9.8 ± 1.3	1.6 ± 0.1
Gastrectomized	5	7.92 ± 0.5	39.3 ± 0.8	7.3 ± 0.6	10.8 ± 1.2	5.9 ± 0.7

*Probable error of the mean.

TABLE V. BLOOD DATA

	DIFFERENTIAL DISTRIBUTION (PER CENT)							TOTAL NEUTROPHILS (THOUSANDS PER C.MM.)
			GRANULOCYTES					
					NEUTROPHILS			
	LYMPHOCYTES	MONOCYTES	EOSINOPHILS	BASOPHILS	MYELOCYTES	METAMYELOCYTES	MATURE	
Prior to Giving Synthetic L. casei Factor in Diet								
Control	66.6 ± 6.3*	1.5 ± 0.1	3.3 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	2.0 ± 0.2	24.6 ± 4.8	2.4 ± 0.7
Gastrectomized	61.6 ± 3.1	0.8	0	0	3.4 ± 0.2	1.2 ± 0.1	33.0 ± 1.1	1.9 ± 0.2
Two Weeks Later: Synthetic L. casei Factor in Diet at Level of 100 Micrograms per Gram; Average Daily Intake: Controls, 1,160 Micrograms and Gastrectomized Animals, 920 Micrograms								
Control	50.0 ± 0.4	0	3.5 ± 0.1	0	7.5 ± 1.6	3.5 ± 0.9	35.5 ± 2.1	4.6 ± 0.6
Gastrectomized	30.7 ± 1.7	0	1.6 ± 0.1	0	10.0 ± 0.4	4.0 ± 1.1	53.7 ± 2.3	7.2 ± 0.7

*Probable error of the mean.

TABLE VI. BLOOD DATA AFTER GIVING SYNTHETIC L. CASEI FACTOR (400 MICROGRAMS) INTRAPERITONEALLY DAILY FOR FOURTEEN DAYS TO GASTRECTOMIZED ANIMALS

ERYTHROCYTES (MILLIONS PER C.MM.)	ERYTHROCYTES (VOLUMES IN CUBIC MICRONS)	HEMOGLOBIN (GM. PER 100 C.C.)	LEUKOCYTES (THOUSANDS PER C.MM.)	RETICULOCYTES (PER CENT)
8.30 ± 0.3*	38.3 ± 1.0	7.0 ± 0.8	14.5 ± 1.3	6.1 ± 1.2

DIFFERENTIAL DISTRIBUTION (PER CENT)							
		GRANULOCYTES					
				NEUTROPHILS			TOTAL NEUTROPHILS (THOUSANDS PER C.MM.)
LYMPHOCYTES	MONOCYTES	EOSINOPHILS	BASOPHILS	MYELOCYTES	METAMYELOCYTES	MATURE	
21.6 ± 2.1	0.2	2.0 ± 0.1	0	22.6 ± 3.4	7.0 ± 1.0	46.6 ± 4.4	11.1 ± 1.1

*Probable error of the mean.

were more marked in test animals than in the controls. Percentages of myelocytes increased from 3.4 to 10.0; of metamyelocytes, from 1.2 to 4.0; and of mature granulocytes, from 33.0 to 53.7. Thus, as a result of providing the *L. casei* factor in the diet for fourteen days, myeloid stimulation produced an absolute increase of total circulating neutrophilic granulocytes from 1,900 to 7,200 per cubic millimeter of blood.

E. Effect on the Blood of Gastrectomized Rats of Injecting 400 Micrograms of Synthetic L. Casei Factor Intraperitoneally Daily for Fourteen Days.—Since we wished to avoid the risk of losing valuable animals, data on the blood were not assembled prior to the intraperitoneal injections of *L. casei* factor. Two weeks had elapsed, however, since the conclusion of the preceding experiment; so that we had reason to believe that the blood picture had reverted to levels previously recorded. The data assembled after fourteen daily injections are condensed in Table VI.

Parenteral administration of the factor was without effect on the total erythrocyte count, the size of erythrocytes, or the grams of hemoglobin per 100 c.c. of blood. There was a slightly greater increase in the percentage of reticulocytes and a considerably greater increase in the total number of leukocytes than occurred when more than twice the amount of the factor was taken with the diet for the same length of time. Again, the total number of lymphocytes per cubic millimeter of blood remained unchanged as a result of these daily injections. However, the percentages of myelocytes and metamyelocytes were both significantly higher, and the total number of neutrophilic granulocytes per cubic millimeter of blood increased from the previous level of 1,900 to 11,100.

It seems obvious from our data that neither the oral nor the parenteral administration of synthetic *L. casei* factor to gastrectomized rats produced a significant effect on the total erythrocyte count, the volumes of erythrocytes, or the grams of hemoglobin per 100 c.c. of blood. It had a decided effect, however, on the myeloid series of leukocytes and its prolonged administration (two weeks) either parenterally or orally resulted in a marked increase of the numbers of immature and mature neutrophilic granulocytes in the circulating blood.

SUMMARY

A study is reported of the effects on the blood changes induced by gastrectomy in white rats, of giving synthetic *L. casei* factor. Sixteen adult male rats were gastrectomized. Of these, six survived for five months and were used as test animals. All were without obvious signs of any gross pathologic changes, as a result of the surgical intervention, but all showed evidence of malnutrition, manifested by progressive loss of weight, and by their rough and discolored coats. Eight nongastrectomized rats served as controls.

Five months after gastrectomy, the total erythrocyte counts were normal, the hemoglobin levels were reduced, and marked microcytosis had developed. Seventy-two hours after a single administration of synthetic *L. casei* factor (200 micrograms), a slight but significant elevation of the percentage of reticulocytes was obtained, but there were no changes in the total erythrocyte counts, the volumes of erythrocytes, the hemoglobin levels, or the total leukocyte counts.

When *L. casei* factor was added to the purified diet, at a level of 100 micrograms per gram, and fed to gastrectomized rats for fourteen days, changes did not occur in the total numbers of erythrocytes, the volumes of erythrocytes, or the levels of hemoglobin. Slightly significant increases occurred in the percentages of reticulocytes and markedly significant increases occurred in the total numbers of leukocytes. This increase of the number of leukocytes was due entirely to a myeloid stimulation, for the numbers of lymphocytes remained unchanged. There was a marked increase of the total number of circulating myelocytes, metamyelocytes, and mature neutrophilic granulocytes.

When *L. casei* factor was injected intraperitoneally daily for fourteen days in amounts equivalent to 400 micrograms, a marked myeloid stimulation likewise occurred. Of the total leukocytes present, 22.6 per cent were myelocytes, 7.0 per cent were metamyelocytes, and 46.6 per cent were mature neutrophilic granulocytes. Thus, of the total number of circulating leukocytes, 76.2 per cent were neutrophilic granulocytes. The parenteral administration of the factor was more effective than providing the factor in the diet.

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PERNICIOUS ANEMIA AND SUSCEPTIBILITY TO GASTRIC NEOPLASMS

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WITHIN the past fifteen years, increasing attention has been drawn to the coexistence of pernicious anemia and epithelial tumors of the stomach. That this association is more than accidental has been demonstrated by a number of large-scale statistical studies of both post-mortem and living cases. Nevertheless, clinical recognition of this fact has been limited and efforts at utilization of pernicious anemia as an "indicator" for the detection of gastric carcinoma in its early stages have been all too few. For this reason, it was thought worthwhile to review the evidence on this subject anew and to discuss its significance in gastric cancer detection programs.

STATISTICAL CONSIDERATIONS

The numerous individual case reports of coexisting addisonian anemia and gastric cancer have been collected by Cotti,[10] Kaplan and Rigler,[28] and others. These have little or no statistical value and will be omitted from this discussion. Similarly, reports of multiple cases of this type have been published by Jenner,[26] Strandell,[54] Cotti,[10] Murphy and Howard,[41] and others. In most of these the anemia had been present for years before the appearance of the gastric tumor, the longest interval being seventeen years in the case cited by Miller.[38] This observation was confirmed by Doehring and Eusterman,[12] who found that the average duration of pernicious anemia prior to the onset of symptoms attributable to the gastric cancer was 8.7 years in their series.

Comparison of the reports from the Mayo Clinic over a period of twenty-five years reveals a distinct increase in the incidence of gastric cancer in patients with pernicious anemia. In the first publication by Giffin and Bowler,[17] only one gastric carcinoma and four other malignant tumors were noted in a series of 628 cases. In the period from 1925 to 1930, Conner and Birkeland[9] found twenty cases of cancer of the stomach among 658 patients with pernicious anemia. Washburn and Rozendaal[62] collected eleven more such cases in the next three-year period. Finally, Doehring and Eusterman[12] reported seventeen additional cases in a series of 1,014 patients with pernicious anemia studied from 1935 to 1939. The greatly increased life expectancy of individuals with addisonian anemia since the introduction of liver therapy is generally offered as an explanation for this apparent increase in gastric cancer morbidity among them.

Not only cancer but adenomatous polyps of the stomach have frequently been reported in association with pernicious anemia.[12, 19, 62] Velde[60] found six such cases (14 per cent) in a series of forty-two patients with pernicious anemia.

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as contrasted with an incidence of 0.71 per cent in a control series of 1,271 patients with other diseases. Such observations assume added significance when the apparently precancerous nature of gastric polyps is considered. In 1930, Miller and associates[39] reported eight personal cases and twenty-four others from the literature in which adenomatous gastric polyps had developed into frank carcinomas. Stewart[53] found carcinoma associated with thirteen of forty-seven gastric polyps, and several other observations of similar character have since appeared.[32, 35, 44] Accordingly, the demonstration of a gastric polyp in association with pernicious anemia may be considered to approach in significance the demonstration of carcinoma per se.

These case reports, even in large series, are merely suggestive of a relationship between the two diseases. A statistical approach is essential to determine whether the frequency with which they are associated is greater than can reasonably be expected on the basis of chance alone. Several studies of this type have appeared, all of which have revealed a frequency of coexistence significantly greater than would have been predicted by probability.

Rambach[43] encountered 641 cases of gastric carcinoma and fifty of pernicious anemia in a series of 11,849 post-mortem examinations. In eleven cases the two diseases were present together, an incidence roughly five times greater than that due merely to chance. Jenner[26] noted five gastric carcinomas, four benign gastric tumors, and only one extragastric malignant neoplasm in a series of seventy-six autopsied cases of pernicious anemia. Brown[5] studied 151 cases of pernicious anemia in a total of 18,200 post-mortem examinations; twelve of these (8 per cent) had gastric polyps and one had a carcinoma of the stomach. The incidence of gastric polyps in the other autopsies is given as 0.003 per cent, which must be an error. However, even when the generally reported autopsy incidence of 0.5 per cent is used for comparison, the number of polyps in the pernicious anemia series is still significantly high.

In addition to the cases found at necropsy, some statistical studies on the morbidity of coexistent pernicious anemia and tumors of the stomach have appeared. Jenner[26] discovered eight cases of gastric cancer in 181 patients with pernicious anemia. He computed the expected morbidity of gastric cancer per decade among the living population of Amsterdam and found that the actual frequency in his pernicious anemia series was twelve times that in the "normal" population of comparable age. Van der Sande[59] similarly calculated morbidity statistics and likewise concluded that the two diseases were associated more often than chance alone would decree. Cotti[10] compiled all of the published case reports in which pernicious anemia had been associated with a malignant tumor and found that the stomach was the primary site in ninety-three of 107 cases, or 86 per cent of the entire series. Since cancer of the stomach represents only about 20 to 40 per cent of all cancers in the general living population, the marked predilection for this organ in patients with pernicious anemia is another link in the chain of corroboratory evidence.

In an attempt to eliminate certain possible sources of error in previous publications on the subject, Kaplan and Rigler[28] studied all cases of pernicious anemia, carcinoma of the stomach, and carcinoma of the colon, cecum, and rectum

recorded in a series of 43,021 consecutive autopsies performed by the Department of Pathology of the University of Minnesota. When corrections were applied for errors in the recorded diagnosis of pernicious anemia, an estimated total of 293 cases of this disease were available for study. Of these, thirty-six also had carcinoma of the stomach, seven had gastric polyps, and four had carcinoma of the colon, rectum, or cecum. Statistical analysis revealed that only twelve cases of carcinoma of the stomach would have been expected. The probability that the observed number was due to chance alone was less than one in one billion. It is apparent, therefore, from all of the foregoing evidence, that some etiologic relationship must exist between pernicious anemia and gastric epithelial tumors.

THEORETIC CONSIDERATIONS

The precise nature of this relationship, however, is still far from clear. On a purely hypothetic basis at least three possibilities can be enumerated: (1) Pernicious anemia directly produces a precancerous state in the stomach; (2) gastric cancer causes pernicious anemia; and (3) the two diseases are linked through a precursor or manifestation common to both.

It seems very unlikely that pernicious anemia is a direct cause of gastric tumors, although conclusive proof to this effect is lacking. That the disease is often in remission at the time when the cancer appears is a common observation, and Dyke and Harvey[13] observed several patients in whom adequate anti-pernicious anemia therapy for a number of years did not prevent the development of gastric carcinoma. The incidence of gastric cancer does not appear to be elevated in patients with other types of anemia, which suggests that anemia per se is not an important etiologic factor.

If cancer of the stomach were caused by exogenous agents in the diet, acting while in direct contact with the gastric mucosa, then the observation by Jacobson and Palmer[25] of prolonged gastric emptying time in patients with pernicious anemia in relapse might throw some light on the association of the two diseases. Although Roffo[46] has reported the induction of gastric tumors in rats fed heated fats or cholesterol, Kirby[29] and others have been unable to corroborate these results, and gastric carcinogens in the human diet are still hypothetic.

Cayer and associates[6] studied vitamin levels in patients with pernicious anemia and found that the levels of nicotinic acid, riboflavin, and thiamin were all significantly reduced. Although vitamins play a selective and important role in the experimental induction of liver cancer, their possible relation to gastric cancer development is entirely unknown.

Likewise, the possibility that gastric cancer causes pernicious anemia can be quickly disposed of by recalling that in many instances where the two have been associated, the latter disease was present for a number of years prior to the appearance of the neoplasm. Because of the frequent occurrence, in patients with gastric cancer, of macrocytic anemias superficially resembling pernicious anemia, some confusion on this point appears to have existed in former years.

In a recent hematologic survey of 127 patients with gastric cancer, Oppenheim and co-workers[42] found a state of anemia in seventy-eight, or 64 per cent.

The anemia was macrocytic in twenty-five cases, normocytic in thirty-five, and microcytic in eighteen. Leukopenia was absent in all but one, sternal marrow studies did not show failure of maturation and the other characteristic features of pernicious anemia, free hydrochloric acid was often present in the gastric secretions, and a typical reticulocyte response to intramuscular liver extract could not be elicited in some of these cases. Inasmuch as evidence of extensive hepatic insufficiency had previously been found in many patients with carcinoma of the stomach,[1] these authors were inclined to attribute the macrocytic anemias observed in their series to hepatic damage. No instance of true pernicious anemia was encountered in this study, a fact which seems at variance with the evidence previously presented. However, the incidence of pernicious anemia in other large series of gastric cancers, though significantly elevated, has still been only a few per cent. Moreover, in a number of personally observed cases, patients with treated pernicious anemia in remission developed microcytic anemias due to chronic hemorrhage following the appearance of their gastric cancers. In such cases the existence of an underlying pernicious anemia would have been completely masked except for the previous history. It is obvious, therefore, that in the series reported by Oppenheim and associates[42] the few cases of pernicious anemia that might ordinarily have been expected could well have been absent on a chance basis alone or obscured by the development of some complication such as chronic blood loss.

It is, of course, theoretically possible for a diffusely infiltrating cancer to destroy the entire gastric mucosa responsible for the formation of "intrinsic factor" and thus produce "secondary" pernicious anemia. Until recently, the absence of this complication in subtotally gastrectomized patients[23] was difficult to reconcile with the demonstration by Meulengracht[36] that the pyloric portion of the sheep stomach was the site of formation of intrinsic factor. This discrepancy has been resolved by the work of Fox and Castle,[16] who found that the human stomach differs from that of the sheep by the fact that the intrinsic factor is elaborated in the fundic rather than in the pyloric portion. Thus, gastrectomy would have to be *total*, including the entire cardia, in order to produce pernicious anemia as a sequel. In a recent review article, Bethell and co-workers[2] expressed the opinion that all patients surviving total gastrectomy for any prolonged period of time will develop pernicious anemia. Similarly, extensive cancers of the fundus and cardia of the stomach might conceivably cause secondary pernicious anemia, but such instances must be quite rare.

It is apparent, therefore, that the majority of cases in which the two diseases coexist must be explained in some other way. A number of features common to both conditions immediately come to mind as possible links between them. These factors include achlorhydria and achylia, atrophic gastritis, and heredity. A hypothetic diagram representing the possible relationships has been published by Rigler and associates.[45]

Haring[19] suggested that the functional deficiency of the gastric mucosa which results in achlorhydria and achylia also acts as a stimulus to proliferation and finally to neoplasia, in a manner analogous to the development of goiter and adenomas of the thyroid in iodine deficiency states. However, the frequency of

achlorhydria is far greater than that of gastric carcinoma, indicating that it is certainly not a dominant etiologic factor. Bloomfield and Polland[3] closely followed a group of forty-three apparently healthy individuals with persistent histamine-refractory anacidity for a number of years and observed neither gastric cancer nor pernicious anemia in any member of the group.

Gastritis has been considered an etiologic precursor of pernicious anemia.[14, 22, 38] However, Magnus and Ungley[34] carefully studied the stomachs of patients with pernicious anemia and found no inflammatory changes. Simple atrophy of the mucosa in the gastric fundus and corpus was the only consistent abnormality. It has been reported that on serial gastroscopic examination, the atrophy and "gastritis" seen in patients with pernicious anemia in relapse often disappears after adequate liver therapy.[27] It is not likely that an essential etiologic factor in a disease would disappear in this manner. Moreover, Moschcowitz[40] has pointed out that achlorhydria and achylia frequently exist for years in the absence of atrophic gastritis, the latter appearing only after the other manifestations of pernicious anemia become apparent. Thus, the weight of evidence would indicate that gastritis is not necessarily a causative factor in pernicious anemia.

The alternative possibility that gastritis is a precancerous lesion has been expounded for years by Konjetzny[31] and others. The frequent demonstration of gastritis in cancerous stomachs naturally suggests a causal relationship, and Warren and Meissner[61] have pointed out the histologic similarity between the severe epithelial changes in some cases of gastritis and the morphologic appearance of known precancerous lesions elsewhere. Torgerson[58] studied the distribution of gastric cancers in unselected cases and in patients with associated pernicious anemia and found that the proportion of tumors arising in the fundus was distinctly higher in the latter group. Because of the known tendency of mucosal atrophy in pernicious anemia to affect the fundus and corpus more than the pylorus, he felt that this distribution of tumors argued for the precancerous nature of atrophic gastritis. However, such evidence is only circumstantial; the careful studies of Hebbel[20] and Guiss and Stewart[18] have failed to demonstrate that gastritis is more common in cancerous stomachs than in those of other individuals of comparable age. Thus, although we cannot refute the possibility that chronic gastritis leads to gastric cancer, present evidence lends little or no support to this view.

The observation of familial tendencies in both pernicious anemia and gastric cancer indicates that common constitutional or hereditary factors may link the etiologies of the two diseases. Conner[8] first demonstrated the high incidence of achlorhydria in relatives of patients with pernicious anemia. Schwartz[50] reported that 18 per cent of a series of patients with pernicious anemia also had relatives with the disease. Among the numerous individual case reports in which pernicious anemia appeared in more than one generation of the same family are those of Faber and Gram[15] and MacLachlan and Kline.[33] The evidence for a familial component in the etiology of pernicious anemia has been summarized and discussed at length by Meulengracht[37] and by Moschcowitz.[40]

Pernicious anemia and gastric cancer have been reported separately or together in several members and in more than one generation of many families. The cases of Borovanská-Felklová[4] and Thiele[57] are particularly striking examples of this type. Zancan,[63] in a geneaologic study of seventy-four patients with pernicious anemia, found evidence of nine carcinomas among their blood relatives, of which six arose in the stomach. He traced a parallel series of relatives of patients with gastric cancer and found that their incidence of gastric cancer was actually lower than that of the group related to patients with pernicious anemia. While such observations are not statistically conclusive, present evidence, though inadequate, favors the thesis that the two diseases are linked through common hereditary influences.

Ivy[24] recently suggested that a study of blood groups in patients with these coexisting diseases and in their blood relatives might yield further information along these lines. In his opinion, the fact that the gastric mucosa is a known storage site for the blood group-specific agglutinins may even have a direct bearing on the incidence of gastric cancer.

One additional hypothesis remains to be discussed. The increasing number of case reports of coexisting pernicious anemia and gastric cancer since the advent of liver therapy was attributed by Saltzman[48] to the longer survival period of patients with pernicious anemia, a view that has been generally accepted. However, Teufl[56] rejected this explanation and speculated that liver extract itself might contain a weak carcinogenic agent which on prolonged administration could lead to cancer in susceptible individuals. This rather startling suggestion finds some experimental support in the work of Kleinenberg and associates,[30] Steiner,[52] and several others who have shown that certain fractions of the livers of both cancerous and noncancerous individuals, when injected into mice or rats, will elicit malignant neoplasms at the site of injection in a small percentage of animals. It has been demonstrated by Strong and coworkers[55] that animals genetically selected for resistance to the local effects of a carcinogen may exhibit secondary susceptibility to the same agent at a remote site. In the light of such evidence, the possible carcinogenicity of therapeutic liver extracts may well deserve further study.

THE EARLY DETECTION OF GASTRIC CANCER

Recent advances in surgery have made possible the salvage of at least 25 per cent of patients with early, operable cancer of the stomach. Refinements in gastroscopic and roentgenologic technique have greatly improved the accuracy of diagnosis of such lesions. Were it not for the fact that early gastric cancer is generally asymptomatic, a considerable proportion of patients now dying of this condition could be saved. Thus, the chief problem in gastric cancer is becoming, more and more, its detection on a practical scale in asymptomatic individuals.

The most obvious way to attack this problem is to examine routinely, by roentgenologic or gastroscopic methods or both, all patients in the gastric cancer-bearing age. However, morbidity statistics compiled by Collins and associates[7] indicate that at any one time only three individuals per 1,000 in the

living population over 40 years of age would be likely to have a carcinoma of the stomach. On the basis of these figures, any mass survey in which no selection of patients was employed would entail an enormous expenditure of time, effort, and money with a very small yield of tumors. This prediction has been confirmed by the work of St. John and co-workers.[47] These investigators, using a rapid fluoroscopic technique, examined over 2,400 asymptomatic individuals past 50 years of age and discovered only two carcinomas and one lymphosarcoma of the stomach. More recently, Dailey and Miller[11] studied 500 apparently healthy men 45 years of age or over and encountered no malignant tumors of the stomach. Such results emphasize the necessity of *selecting* patients for routine periodic examinations of the stomach on the basis of some pre-existing disease, symptom, or other criterion with which the occurrence of gastric cancer can be shown to be associated more frequently than in the general population.

To date, the only known indicator of this type is pernicious anemia. Two years ago, Rigler and associates[45] presented the results of serial roentgenologic and gastroscopic examinations in a series of patients with this disease. At that time, 211 patients had been examined one or more times; of these, seventeen (8.0 per cent) were found to have carcinoma of the stomach, and fifteen (7.1 per cent) had benign polyps. Since then, forty-eight new cases of pernicious anemia have been examined at the University of Minnesota, yielding one additional gastric cancer and two individuals with benign polyps. The summarized data are given in Table I. Comparison of these results with the morbidity, previously cited, of gastric cancer in unselected cases of comparable age reveals clearly the striking "concentration" of carcinomas of the stomach in patients with pernicious anemia.

TABLE I. CLINICAL RESULTS

TOTAL NUMBER OF PATIENTS WITH PERNICIOUS ANEMIA EXAMINED	CARCINOMA OF STOMACH FOUND	BENIGN POLYPS OF STOMACH FOUND	TOTAL TUMORS OF STOMACH FOUND
259	18 (6.9%)	17 (6.6%)	35 (13.5%)

Although no large-scale program of gastric cancer detection has yet been established at New Haven Hospital, even a casual survey of patients with pernicious anemia followed for a reasonable period of time reveals that several have developed polyps, and carcinomas are known to have appeared in at least two such patients, one of whom is alive and well almost two years after gastrectomy.

Unfortunately, patients with pernicious anemia represent a very small fraction of those susceptible to gastric cancer. Other indicators must be determined by careful clinical studies on large groups of variously categorized patients. A large-scale investigation of this type is now in progress at the University of Minnesota, the preliminary results of which were recently reported by State and Wangensteen.[51] Achlorhydria, chronic gastritis, gall bladder disease, and anemia have all been used as criteria for the selection of

patients in this study, and relatives of patients with gastric cancer are also being serially examined. It is to be hoped that the final data will permit the nationwide establishment of selective gastric detection programs from which a high yield of early tumors may confidently be expected.

SUMMARY AND CONCLUSIONS

1. Statistical analyses reveal that pernicious anemia and carcinoma of the stomach develop in the same individuals more often than would be expected on the basis of chance alone and must, therefore, be etiologically related.

2. The evidence pertaining to the nature of this relationship is still inconclusive but suggests that the two diseases are probably linked indirectly through the medium of some common factor. Among the possible factors to be considered are hereditary or constitutional tendencies, achlorhydria, gastritis, and liver therapy.

3. In contrast to the paucity of gastric tumors discovered in nonselective mass surveys, serial gastric examination of individuals with pernicious anemia has yielded a very high incidence of benign and malignant epithelial tumors of the stomach.

4. It is suggested that a search for other conditions etiologically related to or associated with gastric cancer is a necessary prerequisite to the establishment of practical, *selective* mass surveys for the early detection of neoplasms of the stomach.

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THE RADIOSENSITIVITY OF ERYTHROBLASTS

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ALTHOUGH it is generally agreed that the bone marrow may be made extremely aplastic as a result of irradiation, opinion has differed as to the relative sensitivities of the various cell lineages. The attention of most investigators seems to have been directed to a study of the effect of irradiation on the granulocyte precursors. Heineke (1903, 1905), in his classical paper on the effects of x-rays on bone marrow, saw clearly the destruction of myelocytopoiesis but hardly considered the question of the effect of irradiation on erythropoiesis. However, the great numbers of degenerating lymphocytes which he described were probably degenerating erythroblasts. A number of investigators, including Milchner and Mosse (1904), Aubertin and Beaujard (1905), and Jolly (1924), reported that erythroblasts are more resistant than the other myeloid cells. Krause and Ziegler (1906), Lacassagne and Lavedan (1924), Halberstaedter and Simons (1933), and den Hoed, Levie, and Straub (1938) found that after heavier or repeated treatment, the erythroblasts also show evidence of injury. The divergent opinions on the relative radiosensitivity of erythroblasts are reviewed by Selling and Osgood in Downey's Handbook of Hematology (1938). More recently, in Shields Warren's review (1942), Dunlap concludes: "Similarly, the erythropoietic tissue, according to the majority of observers, shows considerable initial radioresistance, but once it is damaged its ability to recover is limited."

Our own observations on the effect of radiation on the bone marrow of rabbits, rats, mice, and chickens have shown the erythropoietic cells to be among the most sensitive of all hematopoietic cells. The findings which have led us to this conclusion will be given briefly in this report. A more detailed presentation will be found in Chapter VI of our report on the "Histopathology of Irradiation From External and Internal Sources" in the Plutonium Project Reports.

MATERIALS AND METHODS

The effects on bone marrow of total body irradiation by single doses of x-rays, fast and slow neutrons, and of radiations from internal sources (strontium^{89}, barium^{140}, lanthanum^{140}, phosphorus^{32}, sodium^{24}, yttrium^{91}, radium, and plutonium) were studied as part of the program of the Histology Group of the Health Division, Metallurgical Laboratory, University of Chicago. For most of the experiments the species used were mice and rats. In addition, a large number of chickens and rabbits were exposed to x-rays and two series of rabbits to fast neutrons. In general, the dosages used were the LD 50/30 days.* Lower doses also were used, these being decreased until morphologic changes were no

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This work was carried out under contract between the University of Chicago and the Manhattan District, Corps of Engineers, War Department.

*The dose which kills 50 per cent of the animals within thirty days.

longer found. For each experiment a group of animals of the same strain and comparable weight and age was irradiated and sacrificed at predetermined intervals. Untreated controls were sacrificed at the same intervals.

X-rays of 200 kvp were filtered with 0.5 mm. of copper and 1.0 mm. of aluminum (Hagen and Zirkle, 1946). The animals were held in plastic boxes and were rotated at least once during the course of the exposure. The distance varied somewhat with the size and number of animals treated at one time, but it was between 70 and 90 cm., giving from 15 to 11 r per minute.

Fast neutrons were primarily from the Clinton pile (Zirkle and Raper, 1946). Some rabbits were exposed to fast neutrons from the University of Chicago cyclotron (Hagen and Zirkle, 1946).

Slow neutrons were given to mice in boron-lined lead boxes inserted in the Clinton pile tunnels (Zirkle and Raper, 1946).

Internally administered β and γ emitters included sodium^{24}, barium^{140}-lanthanum^{140}, strontium^{89}, and phosphorus^{32} injected intraperitoneally, and yttrium^{91} injected intravenously.

Among α emitters, plutonium nitrate or citrate (Snyder and Finkle, 1946) was administered intravenously to mice and rats, or intramuscularly to mice. Radium from which the radon had been blown off twenty-four hours earlier (Norris, Tompkins, and Finkle, 1946) was given intraperitoneally to mice and rats.

Femurs of rats and mice were opened at autopsy by shaving nearly to the midline with a sharp knife, and the entire bone then was fixed in Zenker-formol. To secure the bone marrow of rabbits and chickens, the bone was chipped from the shaft of the femur and a pencil of marrow was removed for fixation. The tissue was then embedded in nitrocellulose, sectioned, and stained with hematoxylin-eosin-azure II.

RESULTS

Irradiation From External Sources.—After x-irradiation the degenerative changes in the bone marrow were of similar type in the rabbit, mouse, rat, and chicken. With the LD 50/30 days, mitotic activity ceased and hematopoietic cells were destroyed, in every case erythroblasts before myelocytes. In the rabbit, as early as one-half hour after treatment, the erythroblasts were already reduced in number, and the remaining ones were predominantly late forms (normoblasts). Degeneration of hematopoietic cells continued at subsequent intervals, and by twenty-four hours erythroblasts had dropped to a minimum. Myelocytes, disappearing less rapidly and their destruction sometimes preceded by bizarre forms, reached a low point at 9 days. Megakaryocytes apparently suffered only occasional damage the first day, but by 2 days few were left. The debris was ingested by phagocytes, and the depleted marrow was replaced first by a gelatinous material and then by fat cells. Reticular cells were never found damaged. As the cells began to regenerate, there was an increase in mitotic figures, a preponderance of young forms of hematopoietic cells, and a diminution of fat cells.

In rabbits exposed to 400 r of x-rays, erythropoiesis in the bone marrow was again affected earlier than granulocytopoiesis, the results differing from those following the higher dose only in degree of damage and in time of its occurrence. Here fewer of the total number of cells were completely destroyed. Lobing of the maturing erythroblasts occurred abnormally early and was excessive. Megamyelocytes appeared, rivaling the megakaryocytes in size. These matured into giant granulocytes with 8 to 12 lobes to the nucleus and eventually degenerated in the marrow.

In rabbits exposed to 100 r of x-rays, the effects on the bone marrow were much less intense and less persistent than those which followed the 400 r dose. While mitosis stopped almost completely, it had been resumed by 3 hours after treatment and continued through the 14-hour interval at a level slightly above that seen in controls. At 8 hours a number of abnormal mitoses were observed; there were also a few dead cells, mostly erythroblasts, occasional nuclei with clumped chromatin, and rare megamyelocytes. From 14 hours through 21 days these marrows were no different from the controls.

In mice exposed to 350 r of x-rays, well below the LD 50/30 days for this species, the general progression of damage and repair of hematopoietic cells was like that observed in the rabbits, again with depletion first most marked in the erythrocytic series, but with myelocytes involved later. Recovery was indicated at 5 days, and was further advanced at 9 and 14 days.

In rats exposed to 600 r of x-rays, the LD 50/30 days, hematopoiesis, normally similar to that in mice, was almost completely eliminated by 2 days after treatment, the depleted areas filling with gelatinous or fatty marrow, but was nearly restored at the 31-day interval. At 3 hours erythroblasts were markedly reduced in number and there was considerable cellular debris. By 14 hours erythroblasts were almost completely gone. Myelocytopoiesis disappeared rather more slowly but by 2 days was practically absent. Regeneration, observed at 9 days with the return of erythropoiesis, was increased at 14 days with a preponderance of young forms. At subsequent intervals this function appeared normal, except for a possibly significant retrogression in the 21-day specimens. Myelocytopoiesis continued to be depressed until about 31 days.

The chicken bone marrow was affected by all doses from 25 to 1,000 r, the damage being roughly proportional to the size of the dose. Only 800 and 1,000 r resulted in the production of gelatinous marrow, and after these doses no permanent regeneration was observed in the 9- and 4-day duration of these experiments.

In 3-week chicks exposed to 800 r, erythropoiesis was completely removed at 1 hour and granulocytopoiesis was gone at 14 hours. Transient waves of regeneration were seen in the erythropoietic series at 2 to 14 hours and at 2 to 5 days; in the granulocytic series the recovery attempts were observed at 5 hours and again at 1 to 4 days. These recovery efforts were futile, however, and the marrow at 9 days was entirely aplastic. In the 11-week chickens two similar waves of regeneration were seen in the granulocytic series.

The presence of lymphatic nodules in the chicken marrow made it possible, in the same organ, to compare damage to lymphocytes with the effects on erythroblasts and myelocytes. Also, since erythropoiesis is intravascular and myelocytopoiesis is extravascular in the bone marrow of this species, it is less difficult to differentiate between very early forms of these two cell lineages than in the mammalian marrow where the two processes are intermingled.

Certain immature cells in the chick marrow proved more resistant to x-ray treatment than others. Most of the large lymphocytes of the lymphatic nodules

and the few stem cells among the myelocytes were intact at intervals when the small lymphocytes were extensively damaged and the basophil erythroblasts had been totally destroyed.

These differences in susceptibility among cell types were observed not only in the 3-week chicks given 800 r, but also in the 11-week chicks given 800 r and in the 3-week chicks given 400 r. In the older birds the small lymphocytes in the lymphatic nodules were dead at 1 and 3 hours, but the large lymphocytes were intact. All the basophil erythroblasts were dead in the sinuses, while extravascularly many normal myelocytes remained. Following 400 r in the younger chicks, the destruction was less severe but the differences in sensitivity were even clearer than after the higher dose. In the lymphatic tissue of the marrow, only some of the small lymphocytes were dead at ½ hour, and while the few hemocytoblasts present with the myelocytes appeared to be unaffected, all basophil erythroblasts inside the sinuses had been destroyed.

The pattern of degeneration and subsequent recovery of the bone marrow of rabbits exposed to fast neutrons was strikingly similar to that observed after x-irradiation. Three hours after exposure to 117 n* the degeneration of erythroblasts was at its peak; 5 hours later the destruction was still prominent and quantities of debris were being phagocytosed. The damage to myelocytes was not conspicuous until 17 hours and reached a maximum at 26 hours, occurring later than the damage to erythroblasts, as in the x-irradiated animals. Progressive cellular depletion, the development of gelatinous marrow, and the eventual restoration of hematopoiesis followed.

In the bone marrow of mice exposed to fast neutrons, hematopoietic cells were destroyed to a degree related to the size of the dose. Especially after the lowest dose used, 65 n, it was quite clear that erythroblasts were the first of the hematopoietic cells destroyed.

Again, after exposure of mice to slow neutrons (400 arbitrary units), the same sequence of cellular destruction occurred. At the early intervals the bone marrow contained an increasing amount of debris and damaged hematopoietic cells, especially erythroblasts. By 12 hours myelocytes were fewer in number and groups of those present were pyknotic. The depletion increased up to 4 days, with gelatinous marrow as well as greatly widened sinuses replacing the cellular marrow. At the height of depletion the marrow was aplastic, except for spindle cells, plasma cells, some granulocytes, and, in the shaft, a few groups of normoblasts. By 7 days regeneration was well advanced. The sinuses were now only slightly dilated, and granulocytes were markedly increased throughout the marrow, especially in the shaft. Relatively few erythroblasts were present. Megakaryocytes were rare, except for an aggregation of them in one area just below the cartilage plate of one specimen. By 11 days there had been a great increase in hematopoiesis in the epiphysis and metaphysis, as well as in the shaft, with much more of it erythropoietic than before. At 2 weeks regeneration appeared to be complete.

*The letter n signifies a purely arbitrary unit for measuring fast neutrons; 1 n is the amount of neutron radiation which produces a reading of 1 r on a Victoreen condenser-type r-meter equipped with a 100 r chamber. This meter is commonly used for measuring X and γ rays in r units.

Irradiation From Internal Sources.—When administered internally, certain radioactive isotopes, including some which are almost pure β emitters and others which emit primarily α rays, affect bone marrow in much the same way, qualitatively, as does irradiation from external sources, especially x-rays and neutrons. Hematopoietic cells were destroyed, sinuses became dilated, and the depleted marrow became gelatinous. In contrast to the picture of sudden destruction and subsequent depletion of cells after x-irradiation, the depletion resulting from internal treatment occurred more gradually and was accompanied by very little cellular debris, as there was ample time for its removal by phagocytosis before it accumulated in large amounts. Also the effects on the marrow persisted, their duration depending on the rate of decay of the agent. The order of destruction of hematopoietic cells was the same here as after external irradiation, erythroblasts before myelocytes and myelocytes before megakaryocytes. Free macrophages, reticular cells, and fat cells were radioresistant. These differences between the several cell strains in sensitivity to irradiation were obvious in mice treated with strontium^{89}, barium^{140}-lanthanum^{140}, phosphorus^{32}, sodium^{24}, plutonium, and radium, and in rats after strontium^{89} and yttrium^{91}. The location and type of "particle" emitted by the isotope modified the extent but not the quality of the injury. Thus, the short-ranged α particles produced a relatively narrow rim of aplastic marrow around each trabecula in which they lodged. The much longer-ranged β particles produced much broader zones of damage; indeed, the marrow of the long bones of mice became completely aplastic after the deposition of fair amounts of the β emitting isotopes in bone.

CONCLUSIONS

In the bone marrow of mammals exposed to total body irradiation with x-rays and fast and slow neutrons, hematopoiesis is severely depressed, the degree of injury varying with the dose. The changes in the marrow cells after the injection of the radioactive isotopes, especially those that deposit in bone, are much the same as after the radiations coming from external sources. In all cases the erythropoietic cells are more susceptible to damage than the myelocytopoietic ones, and these in turn than the megakaryocytes.

Similar findings were demonstrated in chickens exposed to x-rays. In these animals the intravascular position of erythropoiesis makes the recognition of these cells easy even in hematoxylin and eosin preparations. The erythroblasts are at least as radiosensitive as the smaller lymphocytes of the nodules which are common in chicken marrow. The large lymphocytes are somewhat more resistant than the small ones.

Fat cells, free macrophages, and reticular cells (including those lining the sinuses) seem to be completely radioresistant to the dosage of irradiation we used.

These findings are in general agreement with the observations made by our group in comparing the relative radiosensitivity of the lymphatic versus myeloid cells of the spleen of rabbits and especially of mice and rats.

It is quite probable that those authors who described large numbers of degenerating lymphocytes in mammalian bone marrow after irradiation had confused these cells with degenerating erythroblasts. It may be that the extreme radiosensitivity of erythroblasts has often been overlooked because of the acute changes of the leucocytes in the peripheral blood, in contrast to the minor changes in the long-lived erythrocytes.

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THE STRUCTURE OF THE GIANT CELLS IN THE BLOOD-FORMING ORGANS

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INTRODUCTION

NORMALLY the blood-forming organs, particularly the bone marrow, contain a few scattered giant cells or exceedingly large cells. The megakaryocytes, osteoclasts, and possibly the multinucleated plasma cells belong to this group and play a role in the physiologic processes of the various blood-forming organs in which they are found. More numerous and extremely variegated in structure are the giant atypical mononuclear cells and the polymorphonuclear and multinucleated giant cells frequently observed concomitant with or partaking in pathologic processes in the blood-forming organs. It is the purpose of this paper to make readily accessible brief morphologic descriptions and accompanying photomicrographic representations of both typical and atypical giant cells, much in the manner of an atlas in miniature.

The material and records available at the Army Institute of Pathology and its associated Lymphatic Tumor Registry are admirably suited to this purpose, comprising as they do numerous examples of all the common pathologic processes and most of the rarer ones. Furthermore, the diagnostic identification of the more controversial processes is usually a composite of the studies and opinions of many different pathologists representing diverse schools of thought and including specialists in the fields of hematology and hematopathology as well as general pathology. For example, only those cases were utilized in studying the giant cells in reticulum cell sarcoma of the lymph nodes in which the diagnosis of reticulum cell sarcoma was essentially agreed upon by all the consultants.

In the preparation of this paper the author has made frequent use of Haythorn's[1] exhaustive review of the literature on the giant cells with especial reference to the foreign body group.

MATERIALS AND METHODS

The material available comprised clinical records, autopsy or surgical protocols, and formalin-fixed tissues, with the addition, in many cases, of dry-fixed smears or impressions of the hematopoietic organs. All cases had been studied in hospitals. Paraffin sections were prepared from all formalin-fixed tissues and were stained with hematoxylin and eosin. In instances of disturbances in the metabolism of lipids, various fat stains were used. To meet the requirements of individual cases, Wilder's method for reticulum, MacCallum's stain for bacteria, and Kinyoun's stain for acid-fast bacilli were employed. The dry-fixed smears and impressions were stained by the Romanowsky methods, Wright's stain or, preferably, Wright-Giemsa or May-Grünwald-Giemsa, as employed by Slider and Downey[2] and Kolouch.[3] Impressions are obtained by touching a fresh cut surface of tissue to a clean slide, so lightly that but a single

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layer of cells adheres to the glass. The slide is immediately air-dried without the application of heat and is then ready for staining. The imprint method is preferable to smears because the cells are less likely to be distorted and ruptured in the process.

The photomicrographs were all taken at the same magnification (×2000) so that ready comparison of the size of the various structures could be made by the reader. Account must be taken, however, of the shrinkage due to fixation when comparing the size of cells in sections and imprints. The higher power was employed because a number of the cells herein discussed have been depicted at extremely low magnification or not at all in the literature, although the majority have been excellently portrayed.

OBSERVATIONS

A. The Megakaryocytes.—In 1890 Howell[4] differentiated a new type of giant cell in the bone marrow from the osteoclast type originally described by Kölliker.[5] To this separate class of cells Howell gave the name "megacaryocyte." In 1906 Wright[6] traced the origin of the blood platelets from Howell's megakaryocytes. The morphologic descriptions which follow will serve as a basis for comparison of these cells with the various more or less characteristic giant cells to be found in Hodgkin's disease and reticulum cell sarcoma, structures with which megakaryocytes are closely identified by some observers. For further details of megakaryocytic morphology the reader is referred to the recent reviews by Rosenthal[16] and by Dameshek and Miller.[7]

1. Megakaryoblast.—

Morphologic Characteristics in Dry-Fixed Smears or Imprints: This cell is slightly larger than the myeloblast (hemocytoblast) which it rather closely resembles. The megakaryoblast has a round or slightly irregular nucleus and a thin nuclear membrane. The nuclear pattern is produced by small chromatin particles arranged in a fine sievelike or a dark regularly stippled design, somewhat coarser than that of the myeloblast. The parachromatin spaces (karyoplasm) are characteristically pink and sharply demarcated from the chromatin particles, but the nuclear pattern is generally slightly less leptochromatic than that of the myeloblast. One or more nucleoli may be present. The nuclear-cytoplasmic ratio favors the nucleus. The cytoplasm, which forms a narrow rim about the nucleus, is basophilic and nongranular. I have not included an illustration of this cell, which actually is not a giant cell; however, the papers of Jürgens and Graupner,[8] Strumia,[9] Rohr,[10] and Dameshek and Miller[7] present good colored representations of this cell type.

Morphologic Characteristics in Tissue Sections: The megakaryoblast is slightly larger than the hemocytoblast (Fig. 1). Its cytoplasm shows a lessening in basophilic properties. The nuclear membrane is thicker than that of the hemocytoblast and the chromatin masses are coarser, although a nucleolus occasionally may be made out. This cell can be identified in sections only by the nature of its associates in areas of intense megakaryocytic metaplasia.

Orgin: Ordinarily this cell is derived from the myeloblast (hemocytoblast) (Ferrata and Negreiros-Rinaldi[11] 1915, Downey and Nordland[12] 1939) and from

the reticulum cell (hemohistioblast) (Custer[13] 1933, Downey and Nordland[12] 1939, and Dameshek and Miller[7]).

2. Promegakaryocyte.—

Characteristics in Dry-Fixed Smears or Imprints: The size of this cell varies from that of the megakaryoblast to well over 20 microns in diameter (Figs. 2 and 3). The nuclear membrane is thin; the nucleus is larger than that of the megakaryoblast; and the nuclear pattern may retain the fine chromatin-parachromatin features of the latter, although it is usual for masses of clumped chromatin to make their appearance in a few areas at this stage. The clumped chromatin is presented in Fig. 3, whereas the cell in Fig. 4 has largely retained the megakaryoblastic pattern. The nucleus may be round or nuclear lobulation may begin at this stage. In general, the nuclear texture is heavier and nucleoli are usually absent. The cytoplasm is basophilic, scant or abundant, and often presents a fine azurophil granulation about the nucleus. Frequently, as seen in the excellent figures of Downey and Nordland[12] and Dameshek and Miller,[7] granular, occasionally nongranular, platelet-like forms are to be found at the cytoplasmic periphery. The larger cells of this group (Figs. 4, 5) are actually intermediate between promegakaryocytes and megakaryocytes.

Characteristics in Tissue Sections: In tissue sections the promegakaryocyte is larger than the megakaryoblast; both nucleus and cytoplasm are increased in size, and the nuclear membrane is thicker. The chromatin masses are larger and coarser, and the vesicular appearance of the hemocytoblast is entirely lost. Lobulation of the nucleus may occur (Fig. 6) or the nucleus may remain round but slightly irregular (Fig. 7). If an appropriate fixative, formol-corrosive (Downey[14]), and stain, Giemsa, Wright's or Dominici's (Slider and Downey[2]), are employed a small azurophilic or acidophilic granular area appears in the cytoplasm eccentrically near the nucleus and gradually spreads until the entire cytoplasm becomes granular. Unfortunately, ordinary tissue methods fail to bring out cytoplasmic detail in cells of this series.

Origin: Ordinarily the promegakaryocyte evolves from the megakaryoblast although in intense reticular stimulation it can also be derived from the reticulum cell.

3. Megakaryocyte.—

Characteristics in Dry-Fixed Preparations: The megakaryocyte is usually a very large cell, well over 40 microns in diameter. The nuclear membrane is thick, and the nucleus is large, polymorphous (Fig. 8), and extremely complicated in form. The original chromatin-parachromatin distinction may be all but obscured by the coarsening of the chromatin masses, although at times distinct areas of parachromatin can be observed (Fig. 5). The cytoplasm is abundant, basophilic, and contains numerous azurophil granules. Gradually the azurophil granules form into groups surrounded by clearer areas of cytoplasm. Pseudopodial processes at the periphery of the cell are frequently found to contain these platelet-like formations. For further cytologic details and colored figures of these cells the reader is referred to the works of Rohr[10] and Dameshek and Miller.[7]

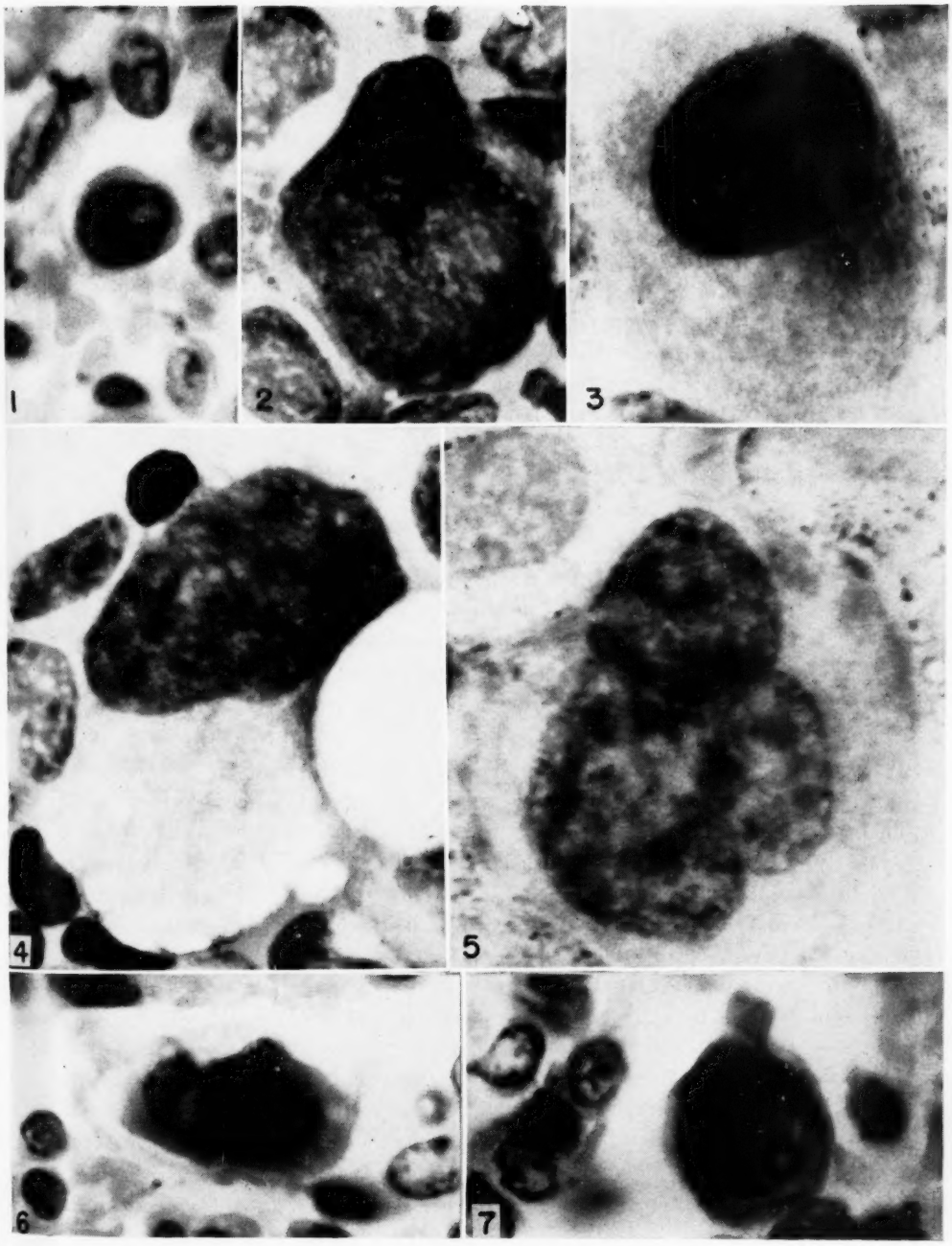

PLATE I

Fig. 1.—Megakaryoblast; section of spleen in myeloid metaplasia. Hematoxylin and eosin stain; ×2000. AIP Neg. 97013.

Fig. 2.—Early promegakaryocyte; sternal bone marrow aspirate. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 96993.

Fig. 3.—Promegakaryocyte; sternal bone marrow aspirate. Wright's stain; ×2000. AIP Neg. 97006.

Fig. 4.—Promegakaryocyte; sternal bone marrow aspirate. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 96992.

Fig. 5.—Intermediate megakaryocyte; sternal bone marrow aspirate. Wright's stain; ×2000. AIP Neg. 97005.

Fig. 6.—Promegakaryocyte; section of spleen in myeloid metaplasia. Hematoxylin and eosin stain; ×2000. AIP Neg. 97015.

Fig. 7.—Promegakaryocyte; section of spleen in myeloid metaplasia. Hematoxylin and eosin stain; ×2000. AIP Neg. 97014.

Characteristics in Tissue Sections: In sections the megakaryocyte may have a diameter as great as 40 microns. The cell outline may be roughly spherical or irregular. The nucleus is large and polymorphous (Fig. 9), the nuclear membrane thick. The chromatin network is composed of coarse dense chromatin strands. Nucleoli are present but usually indistinct. The cytoplasm is abundant and slightly acidophilic. With appropriate fixation and staining, diffuse or grouped azurophil granules are found throughout the cytoplasm. The periphery of the cell is frequently provided with irregular, fingerlike processes of various shapes. In the bone marrow these pseudopodia may be found projecting into the sinuses (Downey,[14] his Fig. 17). Platelets are formed by the fragmentation of pseudopodial cytoplasm, the azurophil granules serving as the chromomere and the fragments of cytoplasm as the hyalomere of the blood platelet.

Origin: Usually the megakaryocyte is derived from the promegakaryocyte, rarely from the polykaryocyte (Di Guglielmo[15] and Rosenthal[16]).

Function: These cells serve to form the blood platelets (Wright,[6] Bunting,[17] and Downey[14]).

4. Dividing Forms of Megakaryocytes.—The cells of the megakaryocytic series reach maturity by means of multipolar mitoses (Rohr[10] and Maximow and Bloom[18]), in which the cytoplasm undergoes no constriction and the daughter nuclei fuse at once into an increasingly pachychromatic, polymorphous nucleus. The fully mature megakaryocyte probably does not undergo further cell division.

5. Polykaryocyte.—Di Guglielmo[15] introduced the concept of the fusion of several histioid elements into a syncytium with multiple nuclei and but a single cell body, which in turn could serve as a source of megakaryocytes. Rosenthal[16] (his Figs. 11 to 13) and Dameshek and Miller[7] (their Fig. 5) depict such cells. The latter authors believe that these cells are probably identical with osteoclasts. Depicted in our Fig. 10 is a multinucleated megakaryocyte which possesses three discrete nuclear masses yet retains the cytoplasmic and nuclear detail of an ordinary megakaryocyte (compare this cell with Figs. 5 and 8). The concept of a polykaryocytic origin of the megakaryocyte has not received general acceptance. Fig. 10 also should be compared with Fig. 47, an osteoclast obtained from a dry-fixed preparation of the bone marrow. The osteoclast, in contrast to the multinucleated megakaryocyte, presents a marked reticulum cell or histiocytic type of nucleus and deeply basophilic cytoplasm lacking azurophil granules.

6. Degenerated Megakaryocytic Forms Encountered in the Normal Bone Marrow.—Upon completion of its platelet-forming function, the cytoplasm of the megakaryocyte, which now has become greatly depleted, disintegrates (Fig. 11). The polymorphous nucleus becomes dark-staining, shrunken, pyknotic, and stripped of almost all of its cytoplasm (Fig. 12). Degenerated megakaryocytic forms are readily confused with the degenerated forms of Reed-Sternberg cells found in Hodgkin's disease (Figs. 26 and 27); therefore, it is imperative that the differential study of these cell types be made before degeneration has progressed to any extent.

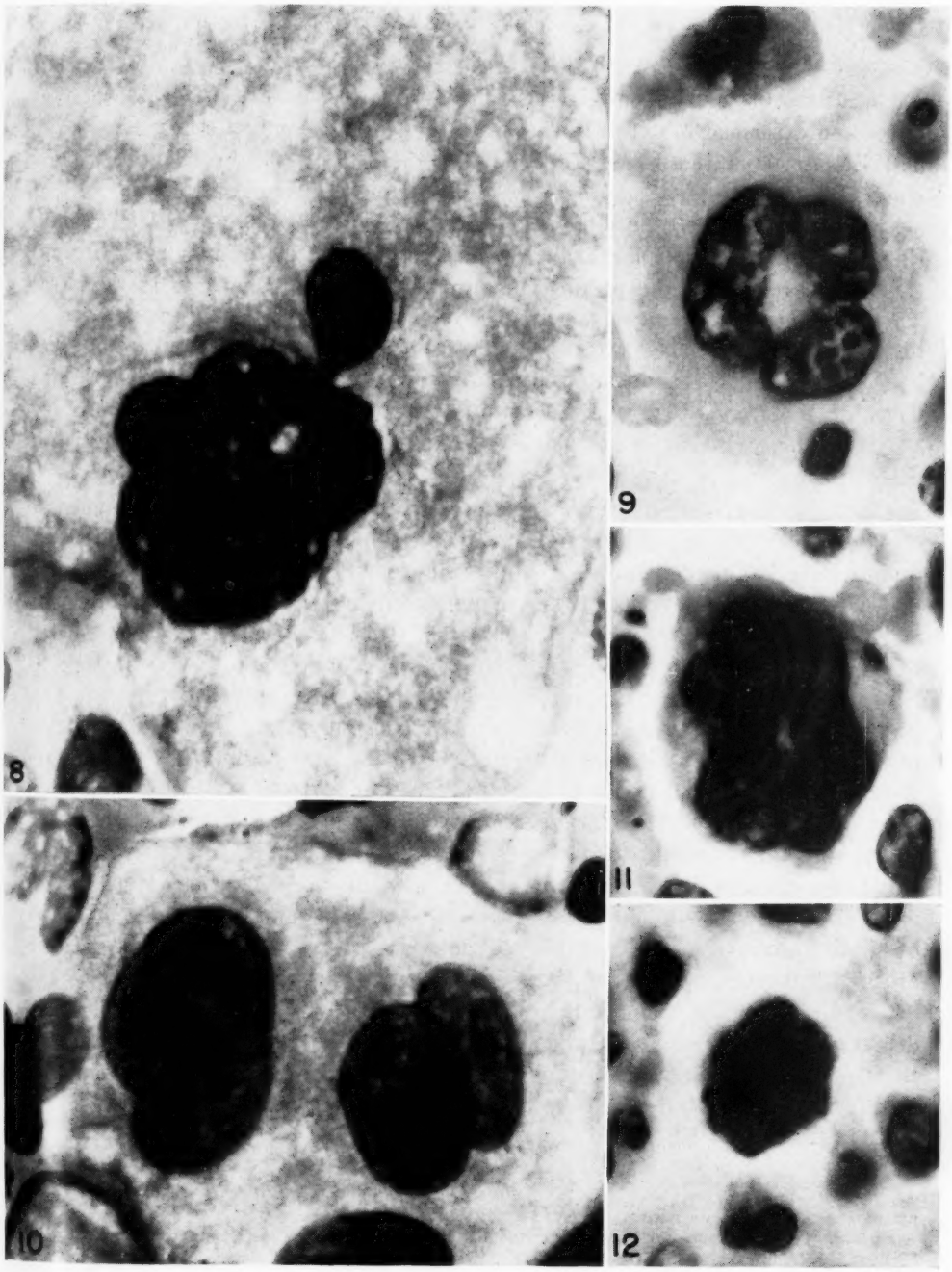

PLATE II

Fig. 8.—Adult megakaryocyte; sternal bone marrow aspirate. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 97614.

Fig. 9.—Adult megakaryocyte; section of spleen in myeloid metaplasia. Hematoxylin and eosin stain; ×2000. AIP Neg. 97243.

Fig. 10.—Multinucleated megakaryocyte; sternal bone marrow aspirate. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 96991.

Fig. 11.—Degenerating megakaryocyte; section of spleen in acute aplastic anemia. Hematoxylin and eosin stain; ×2000. AIP Neg. 97016.

Fig. 12.—Pyknotic megakaryocyte nucleus; section of bone marrow in idiopathic pulmonary fibrosis. Hematoxylin and eosin stain; ×2000. AIP Neg. 97254.

7. Pathologic Megakaryocytic Forms.—

a. In idiopathic (essential) thrombocytopenic purpura the megakaryocytes have been described in detail in the works of Frank,[19] Limarzi and Schleicher,[20] and Dameshek and Miller.[7] In brief, the number of these cells in the bone marrow is normal or increased; many of the adult megakaryocytes present absence of azurophil granulation or decrease in pseudopodial and platelet formation (Fig. 13). The promegakaryocytes show an abortive pseudopodial formation which results in the production of giant, atypical, nongranular platelets. There may be a decrease in the number of megakaryoblasts and an increase in degenerated forms. The cytoplasm of the latter may show vacuolization and hyalinization, and the nuclei hyperlobulation, in addition to the features of degenerated forms already described.

b. In hemorrhagic or hemolytic anemias megakaryocytes are increased but platelet formation is abundant.

c. In hemophilia, polycythemia vera, secondary polycythemia, Gaucher's disease, Felty's syndrome, and aplastic anemia, to mention but a few conditions, quantitative but no qualitative changes in megakaryocytes have been described.

d. In myeloid megakaryocytic hepatosplenomegaly cells in all stages of megakaryocytic development are found in the peripheral blood along with bizarre atypical hypergranular or nongranular platelets. Even the megakaryoblasts and promegakaryocytes present atypical abortive pseudopodial platelet formation, spleen, liver, and lymph nodes may show myeloid metaplasia of a megakaryocytic type, but the marrow is not leukemic and may even possess fewer than the normal number of megakaryocytes (Downey[12]). Paseyro[21] (his Figs. 119 and 122) depicts megakaryocytes in dry-fixed preparations from the spleens of patients with this disease.

e. In chronic myelogenous leukemia any of the conditions existing in myeloid megakaryocytic hepatosplenomegaly may be found, with the addition of similar changes in the megakaryocytic series in the bone marrow and other areas of leukemic "infiltration."

f. In pernicious anemia, Rohr[10] (his Fig. 47) and Paseyro[21] (his Fig. 45) depict marked hypersegmentation of the megakaryocytic nucleus comparable to the hypersegmentation of the neutrophilic leukocytes in the bone marrow in this disease. Jones[22] (his Figs. 10 and 11 and Plate II) found megakaryocytes

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Plate III

Fig. 13.—Mature megakaryocyte lacking pseudopodial platelet formation. Sternal bone marrow aspirate. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 98047.

Fig. 14.—Multinucleated reticular promegaloblasts. Sternal bone marrow aspirate in pernicious anemia. Dr. R. P. Custer's case. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 97885.

Fig. 15.—Three stages of megakaryocytic development. Section of bone marrow in megakaryocytic leukemia. Hematoxylin and eosin stain; ×2000. AIP Neg. 97606.

Fig. 16.—Mature megakaryocyte. Section of lymph node in megakaryocytic leukemia. Hematoxylin and eosin stain; ×2000. AIP Neg. 97897.

Fig. 17.—"Leukemic" megakaryocyte. Section of spleen in megakaryocytic leukemia. Hematoxylin and eosin stain; ×2000. AIP Neg. 97899.

Fig. 18.—"Leukemic" megakaryocyte. Section of bone marrow in megakaryocytic leukemia. Hematoxylin and eosin stain; ×2000. AIP Neg. 97604.

Fig. 19.—"Leukemic" megakaryocyte. Section of spleen in megakaryocytic leukemia. Hematoxylin and eosin stain; ×2000. AIP Neg. 97896.

Fig. 20.—Atypical mitotic figure in megakaryocyte. Section of spleen in megakaryocytic leukemia. Hematoxylin and eosin stain; ×2000. AIP Neg. 97603.

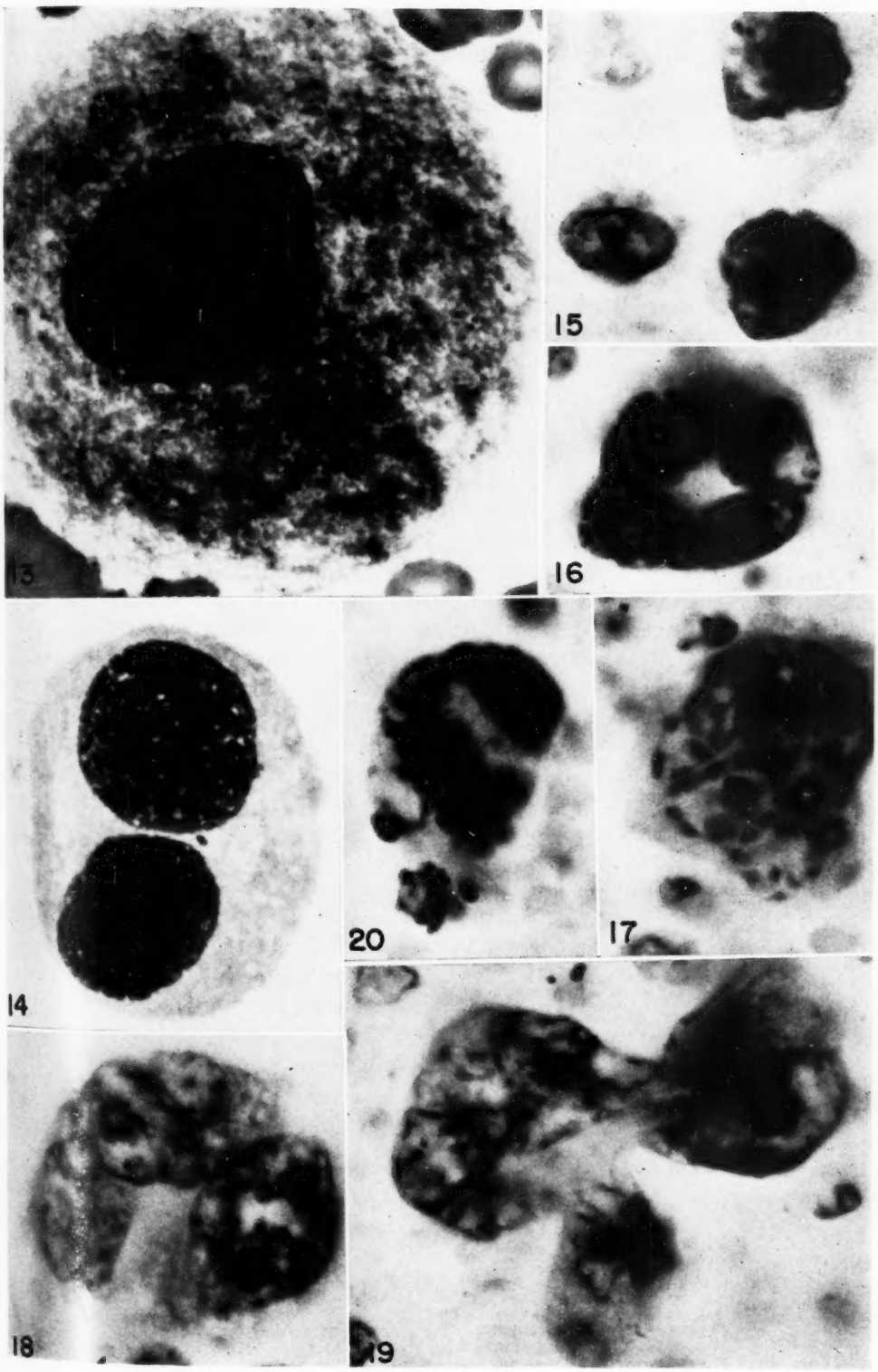

PLATE III

(For legend, see opposite page.)

with "extremely basophilic cytoplasm and mottled areas of hyaloplasm without evidence of platelet formation" in the bone marrow of patients with pernicious anemia in relapse. Paseyro[21] (his Figs. 45 and 46) has noted an intense megakaryocytic reaction in one of his patients with pernicious anemia. It should be noted that in this disease another type of multinucleated giant cell occasionally is found in the bone marrow (our Fig. 14); it is made up of reticular promegaloblasts with multiple discrete nuclei in a single cytoplasmic mass, which stains with the same intense basophilia as does the cytoplasm of individual promegaloblasts. Although the cell illustrated is binucleate, others containing as many as nine nuclei were observed in the same marrow by Dr. R. P. Custer. Rohr[10] (his Fig. 44) illustrates similar reticular-promegaloblastic cell masses in pernicious anemia.

g. In secondary toxic thrombocytopenia, Rohr[10] (his Fig. 102) depicts abnormal and incomplete platelet formation in restricted portions of the megakaryocytic cytoplasm.

h. In megakaryocytic leukemia it is possible to observe what may be termed a "malignant" megakaryocytic series. With the leukemic processes centered in cells of the developing megakaryocytic type, one can compare the structure of the resultant leukemic megakaryocytes on the one hand with definitive megakaryocytes and on the other hand with Reed-Sternberg cells of Hodgkin's disease. The leukemic megakaryocytes arise partly from the hemocytoblasts and partly from the reticulum cells (mesenchymal cells) in the bone marrow, lymph nodes, spleen, and other sites of leukemic "infiltration." Three stages in the early development of these cells in the bone marrow are depicted in our Fig. 15. The structure of the young forms in tissue sections is similar to that already described for cells of the definitive megakaryocytic series. The more fully developed forms, such as the cell from a lymph node in Fig. 16, may likewise closely resemble the definitive megakaryocyte (compare with Fig. 9), although many of them present marked variations. Their nuclei may undergo extreme hyperlobulation (Fig. 17) without degenerative changes, the individual nucleus frequently being massive (Figs. 18 and 19). In spite of these changes it should be noted that the nuclear structure remains vesicular, and if the occasional clumps of coarse chromatin are present, they cling to a moderately thick nuclear membrane (Figs. 17 and 19). Two or three of the giant lobes may contain large abnormal

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Plate IV

Fig. 21.—Atypical enlarged reticulum cell. Section of lymph node in Hodgkin's disease treated with nitrogen mustard. Hematoxylin and eosin stain; ×2000. AIP Neg. 97002.

Fig. 22.—Tripolar mitosis in Reed-Sternberg cell and atypical enlarged reticulum cell. Section of lymph node in Hodgkin's disease treated with nitrogen mustard. Hematoxylin and eosin stain; ×2000. AIP Neg. 97000.

Fig. 23.—Atypical binucleated enlarged reticulum cell. Section of lymph node in Hodgkin's disease treated with nitrogen mustard. Hematoxylin and eosin stain; ×2000. AIP Neg. 97003.

Fig. 24.—Small Reed-Sternberg cell. Section of lymph node in Hodgkin's disease. Hematoxylin and eosin stain; ×2000. AIP Neg. 98052.

Fig. 25.—Large Reed-Sternberg cell. Section of lymph node in Hodgkin's disease treated with nitrogen mustard. Hematoxylin and eosin stain; ×2000. AIP Neg. 97258.

Fig. 26.—Degenerated Reed-Sternberg cell. Section of lymph node in Hodgkin's disease treated with nitrogen mustard. Hematoxylin and eosin stain; ×2000. AIP Neg. 97258.

Fig. 27.—Degenerated Reed-Sternberg cell. Section of lymph node in Hodgkin's disease treated with nitrogen mustard. Hematoxylin and eosin stain; ×2000. AIP Neg. 97257.

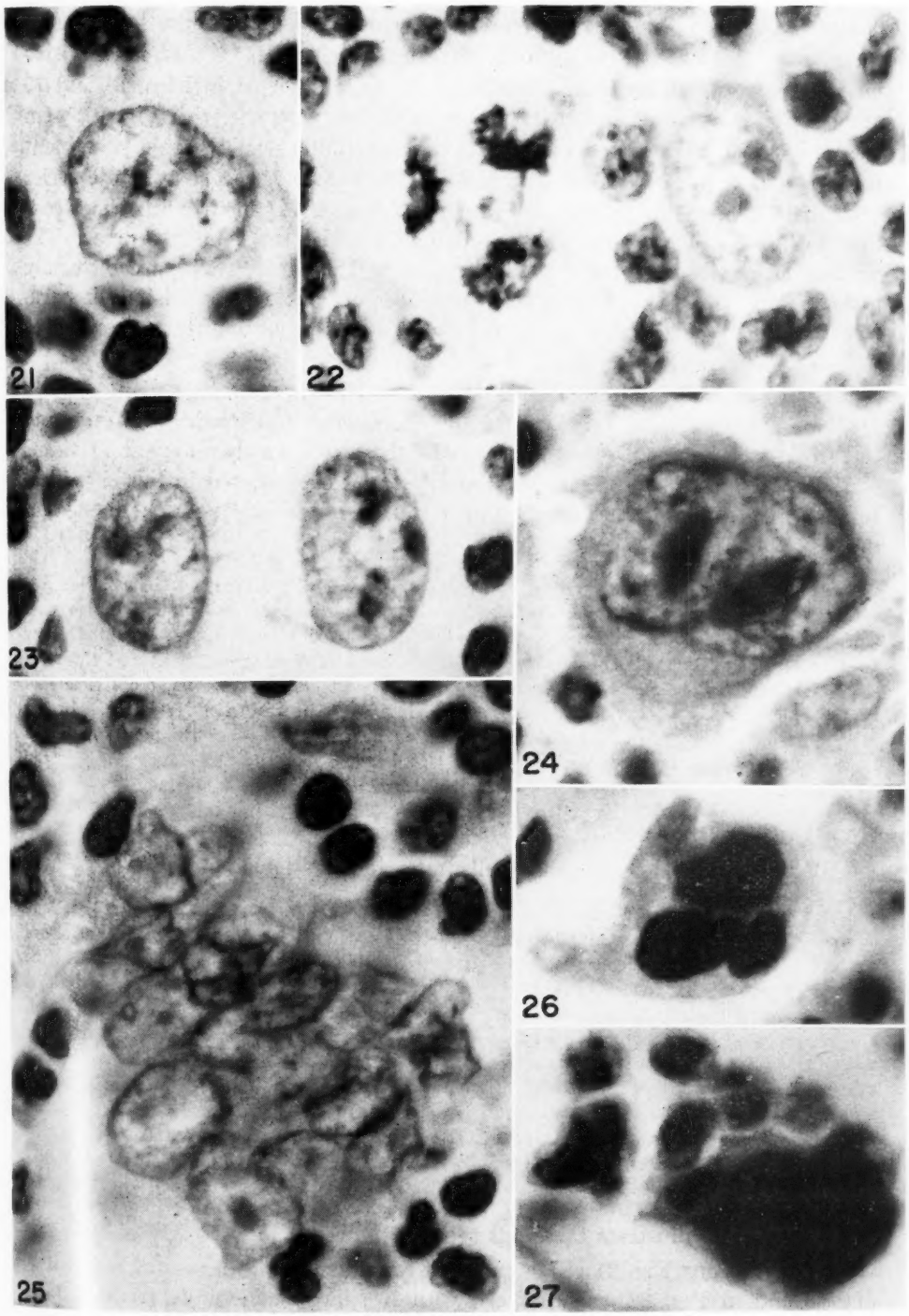

PLATE IV

(For legend, see opposite page.)

basophilic nucleoli; however, they are not a conspicuous feature in the majority of leukemic megakaryocytes, although they are more prominent than in definitive megakaryocytes. Giant bizarre nuclei of the type depicted in Figs. 18 and 19 are not found in definitive megakaryocytic series (compare with Fig. 9). On the other hand, in Reed-Sternberg cells the acidophilic nucleoli are a prominent feature, and cells corresponding to those of the definitive megakaryocytic series (for instance the cell in Fig. 16) are not found in company with the Reed-Sternberg cells in extramedullary malignant lymphogranulomatous areas. The mitoses, as is to be expected in the leukemic megakaryocytic group, are extremely bizarre (Fig. 20). For additional information on cytologic detail it is imperative that such cells be studied by the dry-fixed smear or imprint technique as well as in tissue sections.

B. Giant Cells in Hodgkin's Disease.—The characteristic giant cells in Hodgkin's disease were described first by Sternberg[23] and Reed.[24] Reference to their careful and complete descriptions of these cells as they appear in tissue sections should go far in removing the difficulties encountered in the identification of giant cells of this type. For this reason I shall quote their original descriptions.

Sternberg,[23, p. 23] (1898) writes: "... ferner grössere, protoplasmareiche Zellen mit grossen, dunkelgefärbten Kernen, die nicht so selten mehrfach zu zweien und dreien oder auch gelappt erscheinen. Vereinzelte solche Zellen besitzen eine grössere Anzahl (bis zu fünf und sechs) Kernen. An manchen sind die Kerne auffallend gross, rund und enthalten sich mit Eosin tingierende Kernkörperchen, oder, was seltener der Fall ist, die Kerne sind blass gefärbt und enthalten einen oder zwei sich mit Eosin farbende Nucleolen. In der Mehrzahl sind sie überchromatinreich. Diese Zellen liegen zumeist frei zwischen den Zügen lockeren Bindegewebes, auch denselben an und manchmal so innig und sind auch mit plumpen Ausläufern versehen, dass sie mit dem Stroma in Zusammenhang zu sein scheinen."

His Figs. 1 to 4 illustrate the cells he describes.

In 1902 Reed[24] describes these cells in the following manner:

"The nucleus is always large in proportion to the size of the cell. It may be single or multiple. If single, it is usually round. Bean-shaped and irregularly indented nuclei are common. If multiple, the nuclei may be arranged peripherally in the cell or heaped in the center. Eight or ten nuclei have been seen in a single cell. The chromatin network is prominent in these nuclei, and one or more large nucleoli are always present. The nucleoli are usually oval but they may be of any shape. The nucleoli always take a contrasting stain in the double stains; they have an affinity for acid dyes. No definite mitotic figures were ever seen in these cells. Direct division was frequently observed. The protoplasm is usually homogeneous and stains well. It may appear granular, show vacuolization, or contain fat or pigment granules. Cells having bizarre and irregular nuclei are found in the oldest growths. These giant cells, so far as our observation reaches, are peculiar to this growth and are of great assistance in diagnosis."

Her Figs. 2 to 5 illustrate these cell types.

Shown in our Fig. 21 is the enlarged reticulum cell in a case of Hodgkin's disease and in Fig. 22 a similar cell with two large acidophilic nucleoli, thin nuclear membrane, relatively few particles of chromatin, and scant faintly acidophilic cytoplasm. A very similar but binucleate cell of this type is found in

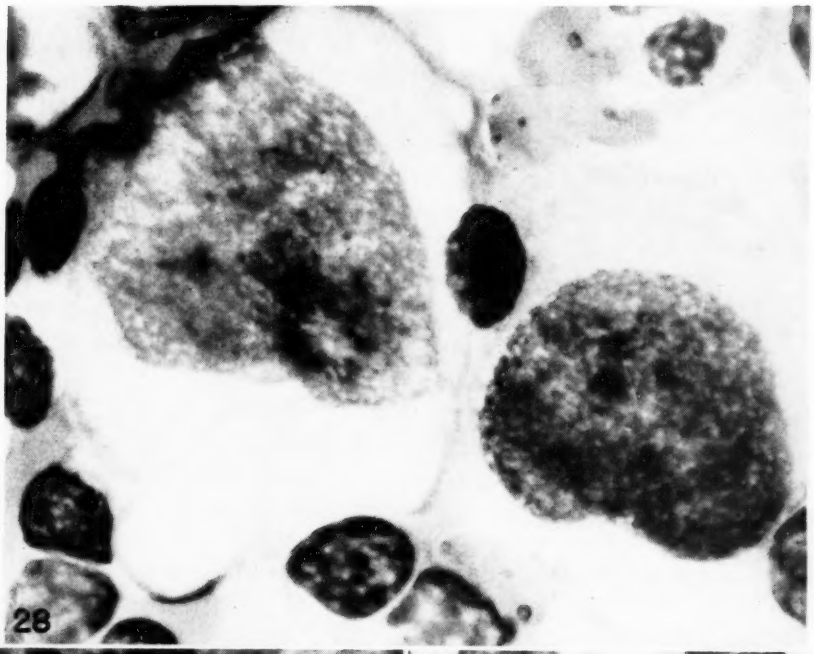

PLATE V

Fig. 28.—Two atypical enlarged reticulum cells. Imprint of lymph node in Hodgkin's disease. Wright-Giemsa stain; ×2000. AIP Neg. 97902.

Fig. 29.—Atypical enlarged reticulum cell. Imprint of lymph node in Hodgkin's disease. Wright-Giemsa stain; ×2000. AIP Neg. 98049.

Fig. 30.—Atypical binucleated enlarged reticulum cell. Imprint of lymph node in Hodgkin's disease. Wright-Giemsa stain; ×2000. AIP Neg. 97903.

Fig. 23. These cells should be compared with Figs. 1 and 7, developing megakaryocytes in tissue sections, which lack the large acidophilic nucleoli and scant chromatin pattern of Reed-Sternberg cells. The simpler form of giant lobulated Reed-Sternberg cell is depicted in Fig. 24. The large round or rod-shaped acidophilic nucleoli, so prominent in these cells, and their relatively moderate chromatin content are in sharp contrast to the nuclear characteristics of intermediate and adult megakaryocytes as seen in Figs. 6, 7, and 9. If further differentiating criteria are required, special fixation and staining (Downey[14]) will reveal the azurophil granulation and granular pseudopodial platelet formation on the part of megakaryocytic cytoplasm in tissue sections, two characteristics which are not features of the cytoplasm of Reed-Sternberg cells. Shown in Fig. 25 is one of the larger bizarre cells of the Reed-Sternberg type with excessive nuclear lobulation. Degenerated forms of Reed-Sternberg cells are a constant feature throughout the course of Hodgkin's disease, and they become even more apparent after the application of the various forms of therapy. Such cells undergoing pyknosis or lysis are shown in Figs. 26 and 27. It is not surprising, when we compare these cells with the degenerated forms of megakaryocytes (Figs. 11 and 12) and note the over-all resemblance between the pyknotic polymorphonuclear and multinucleated forms, that these two entire groups are occasionally identified as one, especially since degeneration may occur at any stage of development of the Reed-Sternberg cell. The observation of Reed[23] that mitoses are never found in these cells has been refuted by more recent findings of multipolar mitoses, as illustrated in Fig. 22.

In the dry-fixed impressions of the blood-forming organs, stained by the May-Grünwald-Giemsa technique, the disparity in structure between megakaryocytes and Reed-Sternberg cells is readily apparent. Paseyro[21] has a comprehensive series of photomicrographs of such preparations (his Figs. 69 to 73 and 75 to 78). In our Fig. 28 is shown the enlarged atypical reticulum cell of this disease. It is comparable to the cell of Fig. 22 as seen in tissue sections. The large size and beginning lobulation of the nucleus become more apparent. The chromatin is arranged in small angular pieces irregularly distributed throughout the nucleus, so as to leave distinct colorless parachromatin spaces. The nucleoli are as yet small, round or irregular, and deeply basophilic. The cytoplasm is abundant and consists of a large amount of flaky, granular, basophilic spongioplasm against a colorless background of hyaloplasm. Very rarely a few scattered azurophil granules are to be found within the cytoplasm, but in only an occasional cell, and the granules are few and never show a tendency to group or clump. Presented in Fig. 29 is a similar giant mononuclear cell with several prominent basophilic nucleoli. Depicted in Fig. 30 is a binucleate cell of the Reed-Sternberg type, lacking prominent nucleoli. Comparison of this illustration with those of the developing megakaryocytes (Figs. 2 and 3), in which the same technique was utilized, reveals a chromatin-parachromatin distinction of the nuclear patterns of the two types but increased clumping of the chromatin particles, absence of numerous or prominent basophilic nucleoli, and beginning azurophil granulation in the cytoplasm about the nucleus of cells of the megakaryocytic series.

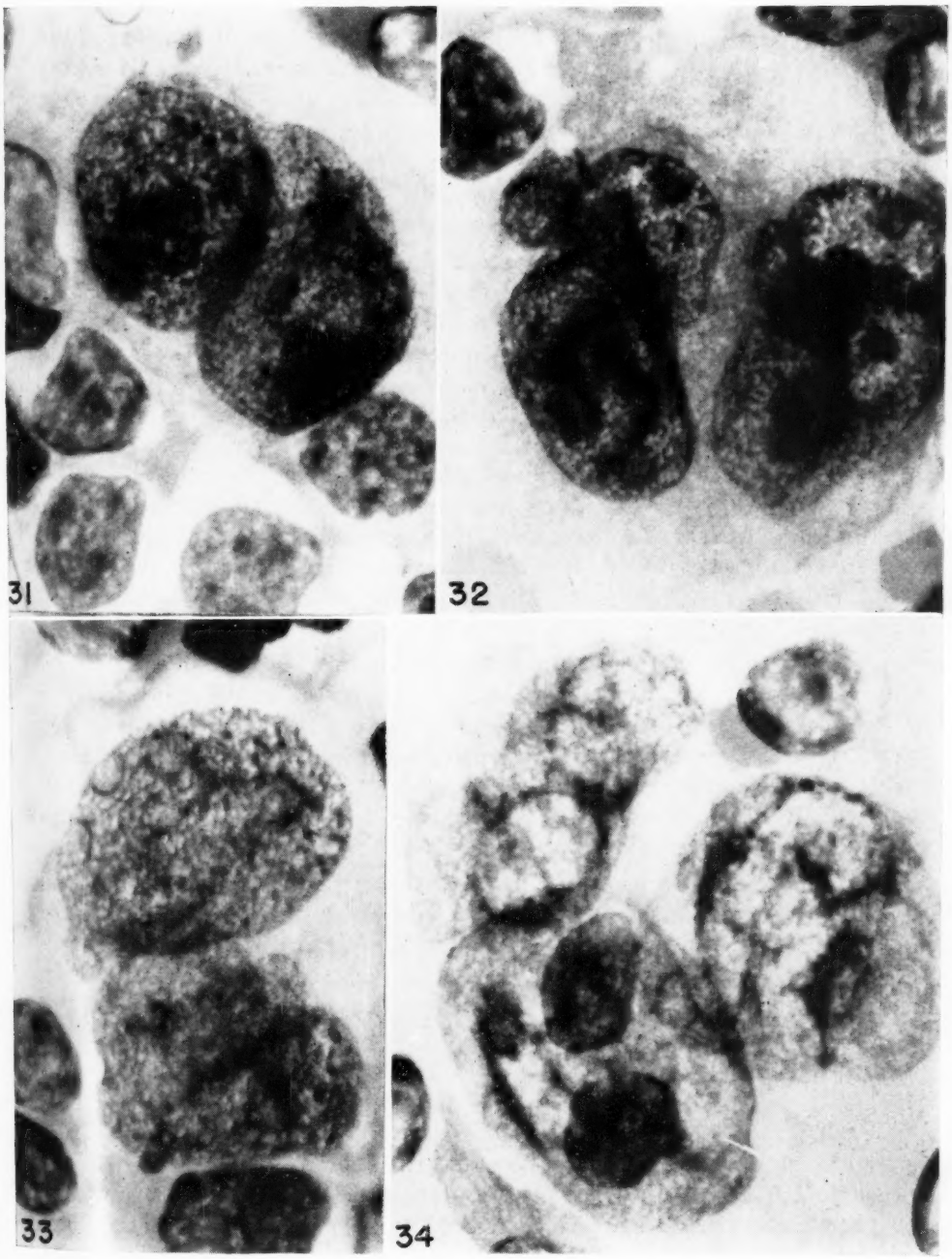

PLATE VI

FIG. 31.—Small Reed-Sternberg cell. Imprint of lymph node in Hodgkin's disease. Wright-Giemsa stain; ×2000. AIP Neg. 98050.

FIG. 32.—Reed-Sternberg cell. Imprint of lymph node in Hodgkin's disease. Wright-Giemsa stain; ×2000. AIP Neg. 97901.

FIG. 33.—Small Reed-Sternberg cell. Imprint of lymph node in Hodgkin's disease. Wright-Giemsa stain; ×2000. AIP Neg. 98048.

FIG. 34.—A part of a large Reed-Sternberg cell. Imprint of lymph node in Hodgkin's disease. Wright-Giemsa stain; ×2000. AIP Neg. 97905.

In Figs. 31 to 34 is revealed lobulation of the nuclei of the Reed-Stern cells, increasing until they become giant, bizarre, and polymorphous. It is to be noted that the fine reticular chromatin network with its chromatin-parachromatin distinction is retained until and beyond the stage of actual beginning cellular degeneration. The nucleoli, which may reach diameters up to 7 microns (Fig. 34), retain their deep, clear, basophilic staining properties. Instead of containing one or two large nucleoli, each nuclear lobe may be spotted with from six to eight small basophilic nucleoli (Fig. 33). The cytoplasm remains as described in the earlier developmental stages. The fully developed Reed-Sternberg cells should be compared with Figs. 5 and 8, with the more mature megakaryocytes, or with the corresponding figures of adult megakaryocytes from the cases of Dameshek and Miller.[7] For the more mature megakaryocytes natural pyknosis has gone on to produce a pachychromatic nuclear pattern; large or numerous basophilic nucleoli are absent or masked, and clumping of azurophil granulation, if not pseudopodial platelet formation, is usually apparent in the cytoplasm. Thus, the value of dry-fixed, imprint preparations is enhanced in cases in which Hodgkin's disease of the blood-forming organs is to be differentiated from those primary or secondary affections of the same organs in which megakaryocytic hyperplasia, "infiltration," or metaplasia is a prominent feature.

Function: The enlarged, abnormal reticulum cells of Hodgkin's disease are rarely found to be phagocytic, according to Jaffe.[25] But at the Army Institute of Pathology we have observed a case in which the Reed-Sternberg cells contained nuclear remnants and others, lipids. In the latter instance cells simulating Touton giant cells were observed, although the central ring of nuclei presented the nuclear pattern typical of Reed-Sternberg cells rather than of histiocytes.

It should also be emphasized that even the earlier authors mentioned nonspecific foreign body giant cells in the primary dyscrasias of the blood-forming organs, in addition to the various specific giant cell forms. Thus, in Hodgkin's disease, Reed[24] describes a "second form" of giant cell, similar to the Langhans' giant cell and Mallory[26] foreign body giant cells. It is interesting that Langhans[27] himself noted the occurrence of cells identical to those which he had observed in tuberculosis, in cases of what he then termed "malignant lymphosarcoma."

C. Giant Cells of Reticulum Cell Sarcoma.—Tumor giant cells appearing in reticulum cell sarcoma have been described by Oberling,[28] Warren and Picena,[29] Gall and Mallory,[30] Foot,[31] and many others. The cells are small for giant cells, usually measuring from 15 to 25 microns in diameter; their cytoplasm is customarily scant and faintly acidophilic in tissue sections, whereas the nucleus consists of a giant multilobulated structure occupying almost the entire cell body. The cells of Figs. 35 and 36 are of this type as seen in tissue sections. The nuclear membrane is distinct and the nuclear lobes are characteristically closely affixed to one another. The chromatin content is sparse, giving a pale vesicular appearance to the lobes, which makes even more prominent a large, round or irregular, basophilic nucleolus to be found usually one or two

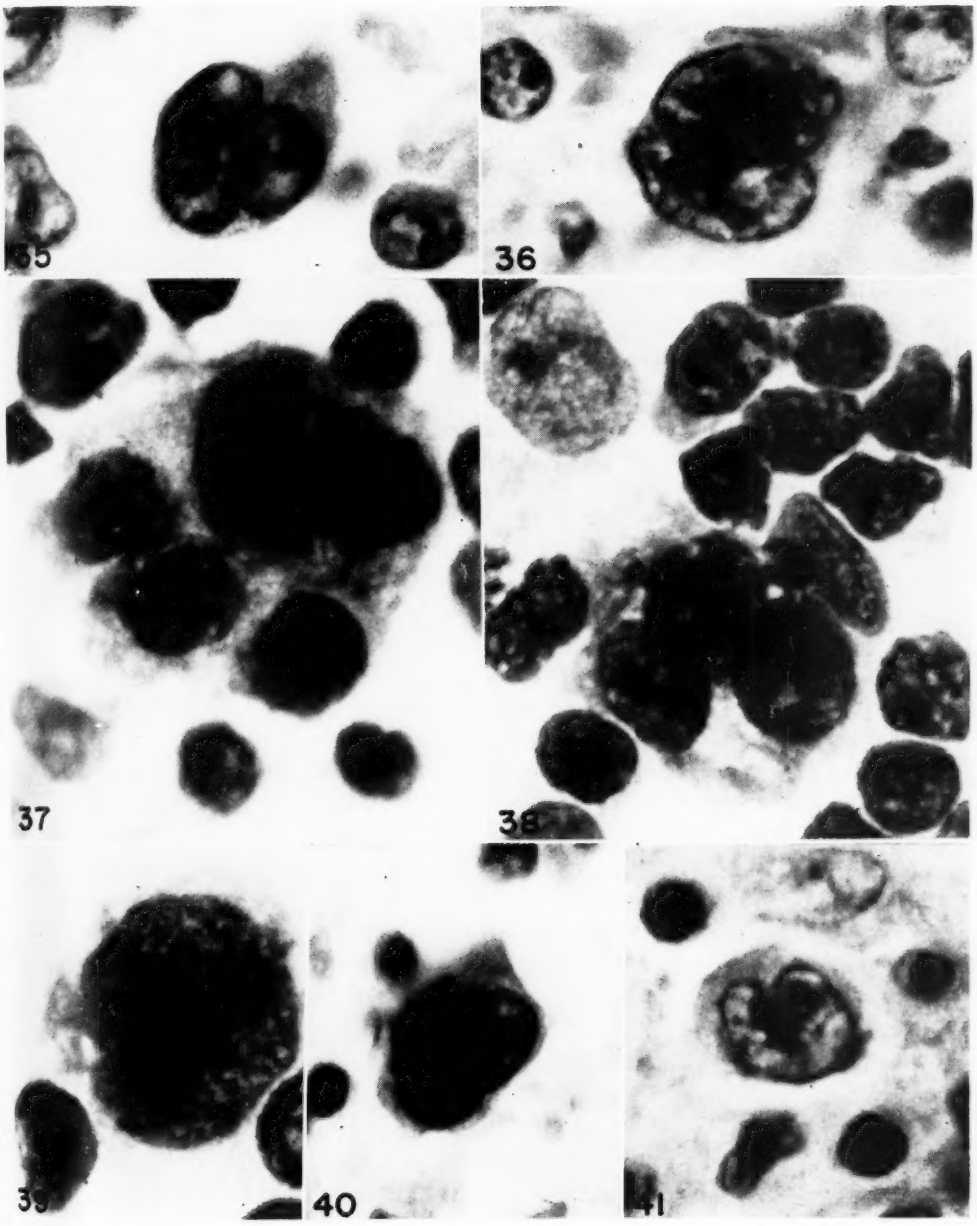

PLATE VII

Fig. 35.—Giant cell in section of lymph node in reticulum cell sarcoma. Hematoxylin and eosin stain; ×2000. AIP Neg. 97616.

Fig. 36.—Giant cell in section of lymph node in reticulum cell sarcoma. Hematoxylin and eosin stain; ×2000. AIP Neg. 98016.

Fig. 37.—Giant cell in imprint of lymph node in leukemic reticulo-endotheliosis. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 97612.

Fig. 38.—Giant cell in imprint of lymph node in leukemic reticulo-endotheliosis. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 97611.

Fig. 39.—Giant cell in imprint of lymph node in leukemic reticulo-endotheliosis. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 97610.

Fig. 40.—Giant cell in section of lymph node in leukemic reticulo-endotheliosis. Hematoxylin and eosin stain; ×2000. AIP Neg. 97609.

Fig. 41.—Atypical enlarged reticulum cell in leukemic reticulo-endotheliosis. Hematoxylin and eosin stain; ×2000. AIP Neg. 97607.

to a lobe. None of these forms contained the acidophilic nucleoli of the fully developed Reed-Sternberg cells as seen in tissue sections.

D. Giant Cells in Leukemic Reticulo-Endotheliosis.—Cases of leukemia in which the leukemic process is centered primarily in the reticulo-endothelial cells as they exert their hematopoietic function, that is, cases characterized by leukemic reticulo-endothelial cells and their differentiation products, have been reported as such by Ewald[32] and Downey.[33] Since cases of this nature are usually included in the literature of the monocytic leukemias, the structure of the leukemic reticulum cells has received little attention from other writers. In several cases of leukemic reticulo-endotheliosis in the material available at the Institute, the leukemic reticulum cells or mesenchymal elements of the blood-forming organs were observed in the process of forming giant, bizarre, polymorphonuclear, or multinucleated reticulum cells similar to those found in reticulum cell sarcomas. In dry-fixed impressions of a lymph node in such a case, cells such as those of Figs. 37 to 39 are seen. The nuclei are round, oval or irregular in outline; the chromatin pattern consists of fine to moderate sized chromatin particles, irregularly arranged, but often sharply demarcated from the parachromatin. The nucleoli are basophilic and small but distinct. Three of the nuclei in Fig. 37 are within the main cytoplasmic mass, the other two apparently are within separate cytoplasmic divisions. The nuclear patterns are less leptochromatic than those of normal reticulum cells, and this may be explained in this case by the fact that differentiation was proceeding along lymphocytic and plasmacellular lines. The cytoplasm may be scant or abundant, and the basophilic spongioplasm is arranged in flakes against a colorless or yellowish hyaloplasm.

In tissue sections the nuclei of these reticular giant cells (Figs. 40 and 41) may possess pale or hyperchromatic patterns. The cell of Fig. 40 was the only one found which resembled the Reed-Sternberg cell; however, the basophilia of the large nucleolus in the one lobe, the hyperchromatism of the remaining lobes, and the small size of the nucleus are not characteristics of Reed-Sternberg cells. Compare these cells of leukemic reticulo-endotheliosis with the illustrations of Reed-Sternberg cells (Figs. 22 to 25) and those of the tumor giant cells of reticulum cell sarcoma (Figs. 35 and 36).

E. Giant Cells in Metastatic Tumors Primary in Other Than Hematopoietic Organs.—Metastatic undifferentiated carcinomas frequently simulate the appearance of primary affections of the blood-forming organs, particularly of the lymph nodes. The tumor giant cells of these metastases, then, must likewise

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Plate VIII

Fig. 42*A*.—Transitional cell carcinoma, metastatic in a section of lymph node. Hematoxylin and eosin stain; ×2000. AIP Neg. 97613.

Fig. 42*B*.—Tumor giant cell, metastatic from breast in a section of lymph node. Hematoxylin and eosin stain; ×2000. AIP Neg. 97009.

Fig. 43.—Tumor giant cell, metastatic from breast in a section of lymph node. Hematoxylin and eosin stain; ×2000. AIP Neg. 97008.

Fig. 44.—Tumor cell in metastatic malignant melanoma in section of lymph node. Hematoxylin and eosin stain; ×2000. AIP Neg. 98013.

Fig. 45.—Tumor giant cell in metastatic malignant melanoma in section of lymph node. Hematoxylin and eosin stain; ×2000. AIP Neg. 98014.

Fig. 46.—Osteoclast in a section of tissue in osteitis fibrosa cystica with a parathyroid adenoma. Hematoxylin and eosin stain; ×2000. AIP Neg. 97906.

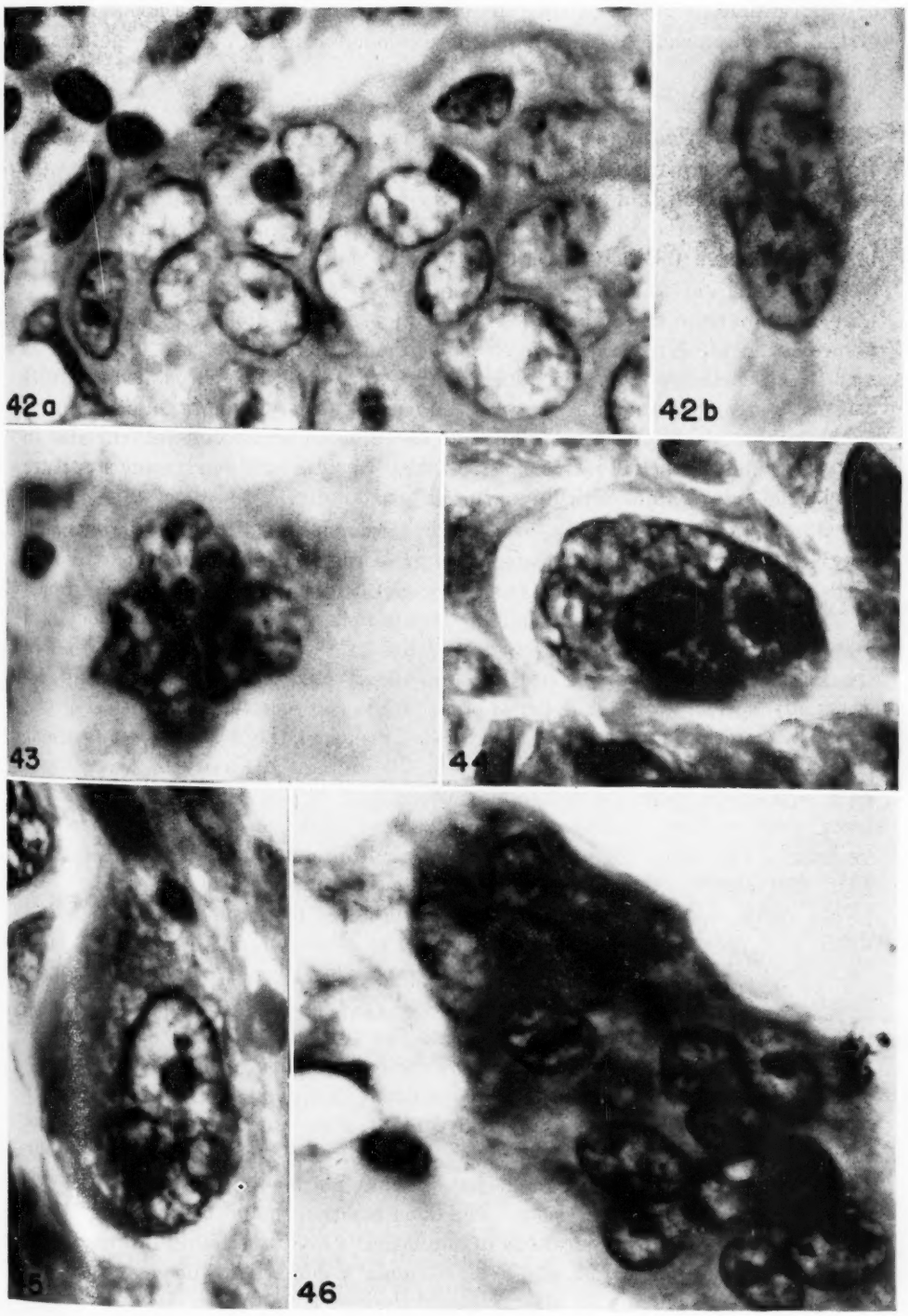

Plate VIII

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be differentiated from the giant cells in primary affections of the hematopoietic system. A discussion of three of the more common examples of these metastases follows.

1. Transitional Cell Carcinoma.—Transitional cell carcinoma (lymphoepithelioma) metastatic to lymph nodes often simulates reticulum cell sarcoma. In Fig. 42a is shown a cord of epithelial cells cut tangentially and apparently surrounded by lymphoid stroma in such a case. Although many of the epithelial characteristics of these cells have been lost, usually enough remain on which to base a definitive diagnosis. Although individual cellular outlines and intercellular bridges are missing, three distinguishing features are listed by Custer[34]; they are: overlapping of the nuclei, prominence and large size of many of the nucleoli, and the syncytial arrangement of the epithelial growth. The nuclei as seen in sections have a distinct nuclear membrane, scant, fine chromatin granules arranged in a delicate linin network, and a rather prominent, round or oval, acidophilic nucleolus. There is marked variation in individual nuclear size in any particular epithelial cord or nest. Ewing[35] points out the alveolar structure and lack of intercellular reticulum after silver staining.

2. Anaplastic Carcinoma.—Anaplastic carcinoma metastatic to lymph nodes may likewise possess morphologic features similar to reticulum cell sarcoma. Figs. 42b and 43 are illustrations of tumor giant cells of an anaplastic carcinoma of the breast metastatic in an axillary lymph node. Usually the functions and hence the special morphologic characteristics of the cytoplasm of the cells in the tissue of primary origin are lost and the cytoplasm is nonspecific, but occasionally special stains will bring out granules or secretory products helpful in the recognition of the true nature of these cells. Otherwise, the tumor giant cells cannot be characterized apart from the extreme diversity of types to be seen in a given lesion. Nuclear lobulations may be few or numerous, nuclear lobes small or large, nuclear outlines extremely bizarre, and nuclei pale and vesicular (Fig. 42b); the more prominent nucleoli are usually acidophilic (Fig. 43). Fortunately, the histopathologic pattern of the metastatic neoplasm, the sheets, cords, or nests of epithelial cells sequestered in the node, is in itself not difficult of recognition. The characteristics of such cells in dry-fixed preparations may be observed in Rohr's[10] monograph (his Figs. 120 and 121a) or as illustrated by Paseyro[21] (his Figs. 30, 33, and 99).

3. Metastatic Malignant Melanoma.—The tumor giant cells of the metastases in tumors of this type present the customary features of the tumor giant cells found in the general group of anaplastic malignancies. Bizarre nuclear outlines, scant or excessive chromatin content, absent or prominent basophilic nucleoli, and moderate or extreme lobulation of nuclei are to be found (our Figs. 44 and 45). Melanin granules may or may not be present within the alveolar type of acidophilic cytoplasm. The dopa reaction should be positive in these cell types. Dry-fixed preparations of similar cells are to be found in Paseyro's[21] monograph (his Figs. 31, 96, and 97). Strumia[9] depicts giant, phagocytic, hemohistioblastic cells laden with black pigment derived from broken down malignant melanoma cells (his Fig. 11 and Plate VIII).

F. GIANT CELLS RELATED TO THE SOFT TISSUES OF BONE.—The giant cells in conditions primarily affecting the soft tissues of bones are included in this discussion, not because of any desire to classify the inflammatory and neoplastic lesions of bones among the disorders of the blood-forming organs per se, but because recent studies of the giant cells in the inflammatory and neoplastic lesions of bones point increasingly to their origin from the same mesenchymal elements that are of intrinsic importance in the origin of primary hematopoietic disorders. It seems reasonable that the structure of such giant cells, falling as they do in a borderline zone between the fields of orthopedic pathology and hematopathology, should engage as much interest among hematopathologists as the reticuloendothelial system does among orthopedic pathologists.

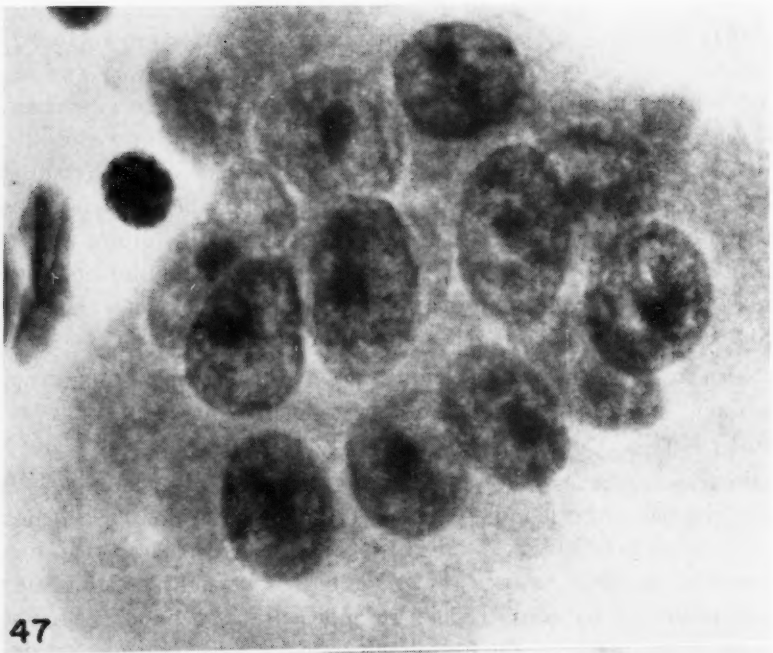
1. Osteoclasts.—These large, multinucleated cells make their appearance wherever bone is formed or undergoes a destructive process. They were first described by Kölliker[5] in 1872.

In tissue sections they are large multinucleated cells, measuring from 30 to 90 microns, with the longer diameter frequently parallel to a bone spicule but sometimes perpendicular to one. The nuclei are round or oval, are rather poor in chromatin, and possess a distinct nuclear membrane and one or two prominent, round, or rod-shaped (Fig. 46) nucleoli. The cytoplasm is abundant, deeply basophilic, granular or vacuolated, and provided with branching processes. Osteoclasts are usually found in Howship's lacunae, bays or grooves in dissolving bone (Fig. 46). Rustizky[36] said that they all contained calcium granules, but Maximow and Bloom[18] found neither bone salts nor cell remnants in their cytoplasm.

In dry-fixed smears or imprints the many round or oval nuclei of the osteoclast resemble the pattern of histiocytic or reticulum cell nuclei, although, again as in sections, one to three prominent, deeply basophilic nucleoli are observed (Fig. 47). The nuclear pattern is characterized by small angular distinct chromatin particles irregularly distributed throughout the nucleus. Here there is chromatin-parachromatin distinction and the nuclear membrane appears to be thinner than in section preparations. The cytoplasm immediately surrounding the nuclei is deeply basophilic and composed of a dense granular spongioplasm, whereas the more peripheral cytoplasm is composed of a background of slightly acidophilic hyaloplasm in which occasional flakes of basophilic spongioplasm appear. No azurophil granules are observed. Sabin and Miller[37] in their Fig. 5 show such an osteoclast in a fixed film of marrow, its cytoplasm filled with eosinophilic particles which stain like bone, and its nuclear configurations difficult to make out.

Origin: These cells arise from the mesenchymal elements of the bone marrow and periosteum, occasionally by fusion of osteoblasts.

Function: Most writers[35, 37] attribute a phagocytic function to the osteoclasts, placing them in the group of foreign body giant cells because of their constant relation to the resorption or dissolution of bone and their disappearance when the resorption of bone ceases in a particular site.



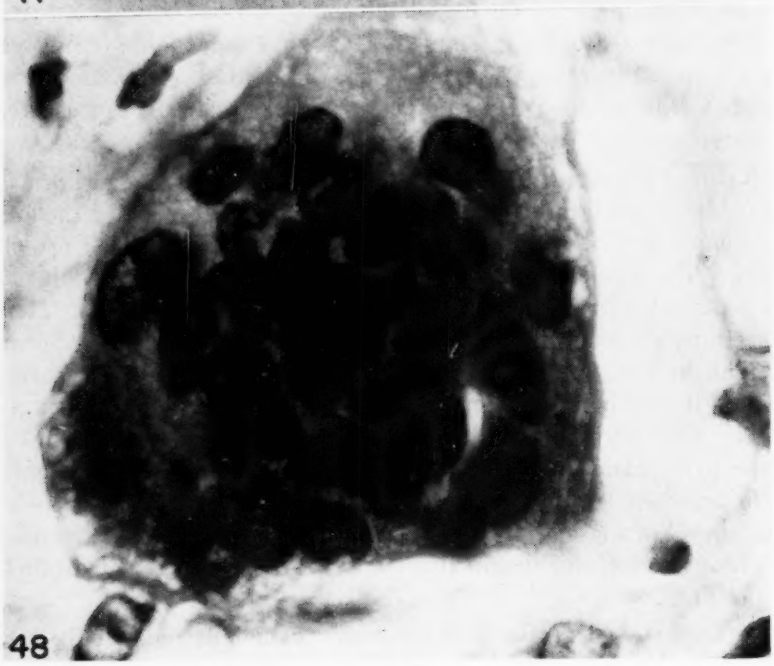


PLATE IX

Fig. 47.—Osteoclast in sternal bone marrow aspirate. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 98023.

Fig. 48.—Multinucleated giant cell in a section of tissue in osteitis fibrosa cystica with a parathyroid adenoma. Hematoxylin and eosin stain; ×2000. AIP Neg. 97884.

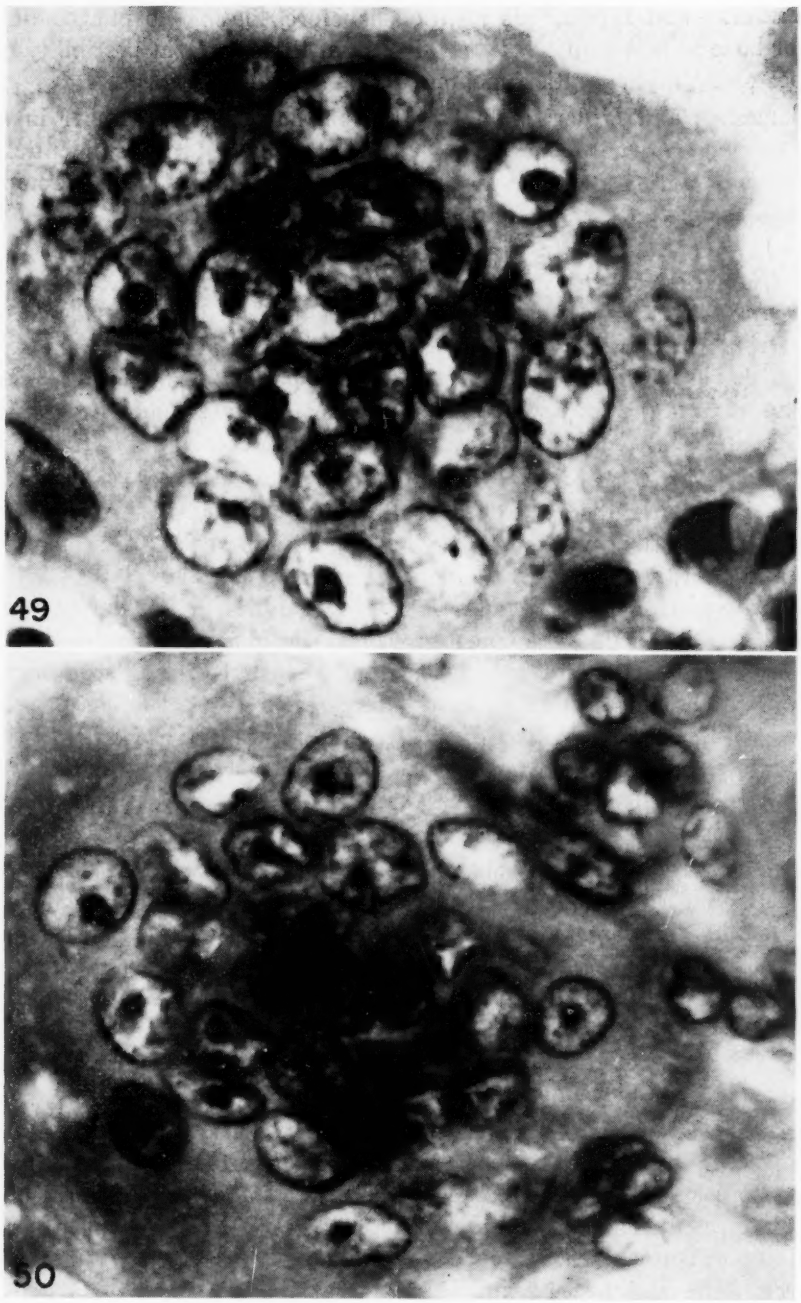


PLATE X

Fig. 49.—Multinucleated giant cell in a section of benign giant cell tumor of bone. Hematoxylin and eosin stain; ×2000. AIP Neg. 97894.

Fig. 50.—Multinucleated giant cell in a section of malignant giant cell tumor of bone. Hematoxylin and eosin stain; ×2000. AIP Neg. 97893.

Maximow and Bloom,[18] as mentioned before, finding no phagocytic inclusions or bone salts within osteoclasts, hold that there is slight possibility of such cells possessing a phagocytic function.

Dameshek and Miller[7] state that the polykaryocyte, precursor in a relatively uncommon type of megakaryocytic formation, is probably identical with osteoclast and forms the adult megakaryocyte by nuclear fusion. (Comparison should be made of their Fig. 5, our Figs. 10 and 47, and Rosenthal's[16] Figs. 11 and 13.)

2. Giant Cells of Bone in Osteitis Fibrosa Cystica Due to Adenoma of the Parathyroid Glands.—Osteoclasts are numerous about the bony spicules in the characteristic lesions of osteitis fibrosa cystica (our Fig. 46). Giant cells, indistinguishable from osteoclasts, are likewise numerous in the fibrous connective tissue apart from the bony spicules or in and about foci of hemorrhage (Fig. 48). However, ingested red corpuscles are not observed within the cell bodies.

3. The Giant Cells of Benign Giant Cell Tumor of Bone.—Numerous giant cells are irregularly distributed through a benign stromal background of connective tissue in this condition. They have to all appearances the same morphologic characteristics as osteoclasts in tissue sections (Fig. 49), but they lack the relationship to Howship's lacunae. Their appearance in imprint preparations has not been described. Many modern writers consider these giant cells as osteoclasts or foreign body giant cells, not as tumor cells. Ewing,[35] however, emphasized giant cell proliferation as an essential feature of the lesion to which he referred as "osteoclastoma." Geschickter and Copeland[38, 39] ascribe these giant cells to abnormal hyperplasia of osteoclasts, whereas Jaffe and associates[40] state that these giant cells are not osteoclasts and that they possess definite differences from osteoclasts.

Origin: These cells probably arise from mesenchymal elements[40] (histiocytes, reticulum cells); however, because these cells at times apparently form a portion of the lining of the blood vessels, an endothelial origin has been claimed, although it should be recalled that many of the simple vascular channels within bone are lined by littoral reticulum cells and not by simple endothelium. Foot[31] tends to favor the idea of a reticulo-endothelial origin of these cells and notes that although the giant cells usually lie in clear spaces amid the reticular network, remnants of argyrophil substance may be found within their cytoplasm.

4. The Giant Cells in Malignant Giant Cell Tumor of Bone.—The stromal background of connective tissue is neoplastic both in structure and functions. The multinucleated giant cells irregularly distributed throughout these lesions (Fig. 50) present few or no characteristic features which distinguish them from osteoclasts. Their appearance in dry-fixed impressions has not been described. However, Jaffe and associates[40] observed that the nuclei of these giant cells actually may share in the atypism of the stromal cells (their Fig. 4) and become swollen and large, out of proportion to cell size. It is important to note that these cells are almost always absent in the metastatic lesions of this tumor. There is but one case in the files of the Institute of Pathology in which the same "benign" appearing, multinucleated giant cells were found in an extension or

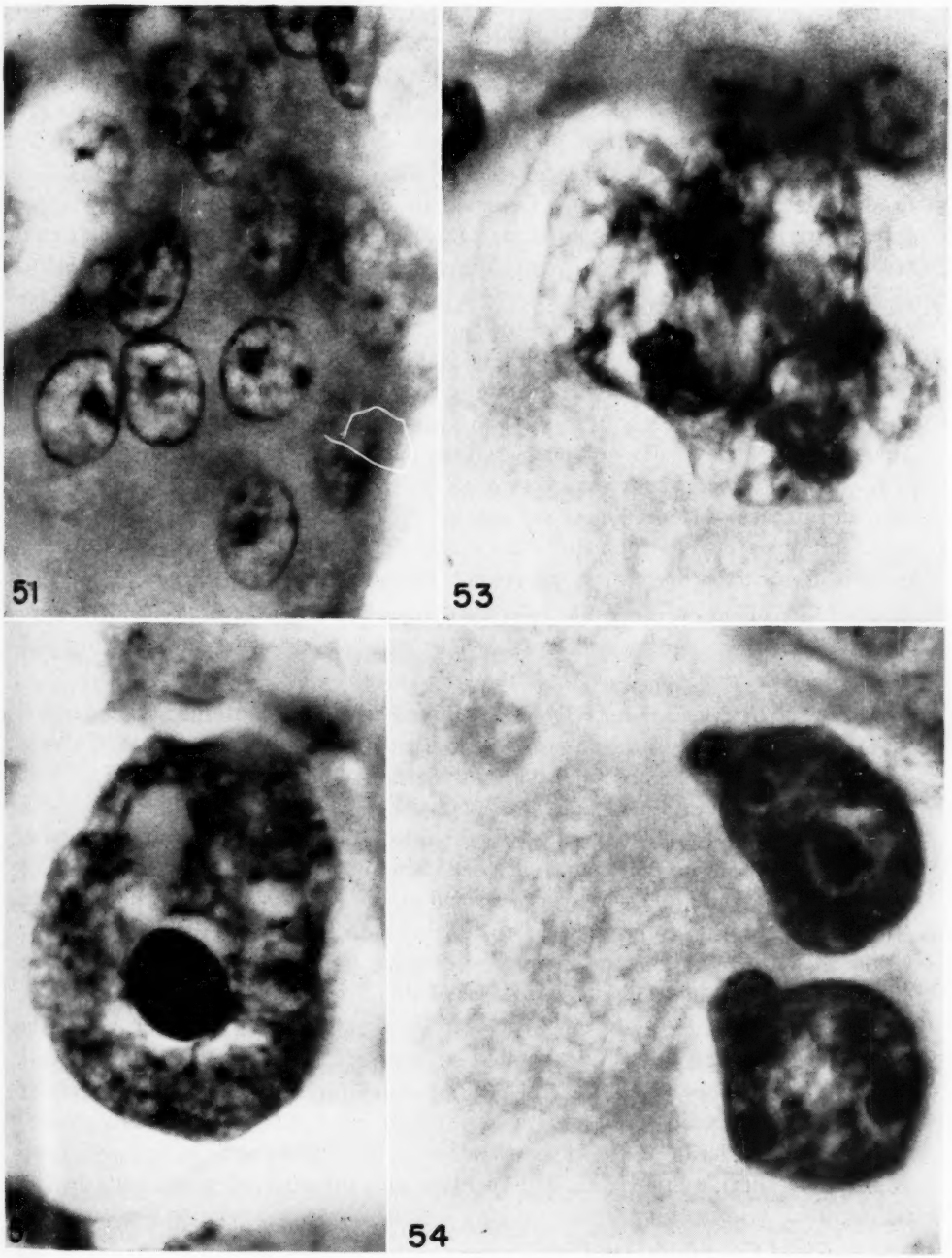


PLATE XI

Fig. 51.—A portion of a multinucleated giant cell in a section of osteogenic sarcoma. Hematoxylin and eosin stain; ×2000. AIP Neg. 97890.

Fig. 52.—Mononuclear tumor giant cell in a section of osteogenic sarcoma. Hematoxylin and eosin stain; ×2000. AIP Neg. 97889.

Fig. 53.—Multilobulated tumor giant cell in a section of osteogenic sarcoma. Hematoxylin and eosin stain; ×2000. AIP Neg. 97892.

Fig. 54.—Binucleated tumor giant cell in a section of osteogenic sarcoma. Hematoxylin and eosin stain; ×2000. AIP Neg. 97891.

spread from the tumor in the ulna to an epitrochlear lymph node. Our Fig. 50 is from the ulnar lesion in this case.

5. Giant Cells in Osteogenic Sarcoma.—In many instances tumor giant cells of all sizes and shapes are abundant in this lesion. These tumor giant cells are to be distinguished both from the foreign body giant cells which may also be present, particularly about areas of hemorrhage, and from the osteoclasts in focal areas of bone resorption. Depicted in Fig. 51 is a giant cell of this reactive type from an osteogenic sarcoma. The tumor giant cells of osteogenic sarcoma may be giant mononuclear forms (Fig. 52), polymorphonuclear forms (Fig. 53), or multinucleated forms (Fig. 54). The extremely large size of the nuclei may be appreciated at once by comparison with those of any of the osteoclasts previously described (Figs. 47 and 48). The nuclei are bizarre in shape, frequently hyperchromatic with coarse chromatin clumps in evidence (Figs. 52 and 54). One or more giant, round or irregular, acidophilic nucleoli are present. The cytoplasm may be scant or abundant and shares in the cytoplasmic type of the more numerous, surrounding, smaller neoplastic cells as to intrinsic structure and nature of intercellular substance. Bizarre, atypical, occasionally multipolar mitoses are to be found in these cases. The morphologic characteristics of the cell in dry-fixed preparations have not been described.

Origin: Tumor giant cells arise by abnormal mitoses in or fusion of tumor cells. Ultimately they arise from the embryonic or mesenchymal elements capable of bone formation.

6. Giant Cells in Ewing's Tumor of Bone.—Ewing[35] mentioned an occasional report of the presence of giant cells in the tumor known by his name. This observation is of importance to the hematologist in view of Piney and Hamilton-Paterson's[41] description of giant, histiocyte-like elements, which they call "dysmorphokaryocytes." These cells, which possess indented nuclei and irregular hyaline cytoplasm, may be found scattered among the cells of the sternal marrow prior to any other indication of the spread of the tumor from its primary state. No example of this cell type was to be found in the material which we have studied. Rohr[10] states that in his hands sternal punctures have revealed no sure signs of malignant changes in cases of this disease.

Origin: Ewing[35, p. 368] suggested as the cellular origin of his tumor "the perivascular endothelium," which to him represented a perivascular cell with mere endothelial potentialities. Oberling and Raileanu[42] and more recently Stout[43] would prefer to designate this tumor as a variant of reticulum cell sarcoma on the basis of the demonstration of reticulum cells as integral parts of some of them. Stout does not mention the presence of giant cells in this tumor.

7. Giant Cells in Other Conditions.—Giant cells identical with or similar to the osteoclast or the foreign body type may also be found in healing fractures, exuberant callus formation, acute and chronic osteomyelitis, rickets, osteomalacia, osteogenesis imperfecta, osteochondritis desiccans, Paget's disease, angioma, fibrous dysplasia of bone, medullary fibroma, xanthofibroma, and medullary chondroma, to mention but a few conditions affecting the soft tissue of the bone and not ordinarily the province of the hematopathologist.

G. Plasmacellular Giant Cells.—

1. Multinucleated Plasma Cells.—Multinucleated plasma cells usually are present wherever the ordinary mononuclear plasma cells are to be found in the blood-forming organs. They were described first by Cajal (Michels[44]). They are not uncommon in practically any of the subacute and chronic inflammatory reactions as well as in agranulocytosis and aplastic anemia (Rohr's[10] Figs. 22 and 71).

In stained tissue sections they are smaller than most giant cells, being about 20 microns in diameter, round, oval or elongated, and possessing from 2 to 4 discrete nuclei usually placed near the periphery of the cell. At times the nuclear pattern may be rather characteristic, consisting of coarse particulate chromatin arranged about the lining of the nuclear membrane in a spokelike fashion so as to present Pappenheim's "radkern" effect; in numerous instances the pattern may merely resemble the lymphocytic nuclear pattern with somewhat coarser features. A nucleolus usually is present. The cytoplasm is occasionally vacuolated and is deeply basophilic, save for a central pale area near the nuclei which is a rather specific characteristic. When stained with Pappenheim's methyl green-pyronin the cytoplasm is a brilliant red whereas the chromatin is a greenish purple (Slider and Downey[2]).

In dry-fixed impressions the nuclei are round or oval and possess a chromatin pattern midway between those of the reticulum cell and the lymphocyte, in that the chromatin granules are arranged in coarse clumps but present chromatin-parachromatin distinction (our Fig. 55). Characteristically in these preparations the "hof" or central light area has disappointingly vague borders and the cytoplasm is found to consist of closely packed, deeply basophilic granules or flakes at the periphery; these become increasingly scant as the center of the cell is approached, allowing more and more of the pale pink hyaloplasm to show through in the central cytoplasmic area. These cells are devoid of azurophil granules.

Origin: These cells arise from pre-existing plasma cells by amitotic nuclear division without cytoplasmic division (Michels[44]) ultimately from reticulum cells or lymphocytes.

2. Multinucleated Myeloma Cells.—Because of our lack of knowledge as to the function of the plasma cell, it is difficult to affirm or deny Rohr's[10] contention that multiple myeloma represents a malignant tumor of the reticulum. However, certain differences in structure between the cells of this and the preceding group can be noted at times.

These cells as seen in tissue sections are usually merely binucleate, but 3 to 8 or even more discrete nuclei have been observed. They may resemble plasma cells with their round, lymphocyte-like, peripheral nuclei and abundant basophilic cytoplasm with a central light area (Fig. 56), or they may present all transitions to larger cells with more abundant colorless or slightly acidophilic cytoplasm, the pale perinuclear area thus being lost. Ewing[35] observed mitoses in these cells but less frequently as they approached the plasma cell type. According to Jones,[22] myeloma cells do not take the specific methyl green-pyronin stain described for plasma cells.

In dry-fixed preparations the discrete nuclei may vary in size within a single cell and in different cases may show nuclear patterns of the reticulum cell, the plasma cell, or the lymphocyte. These cells may possess as many as from 5 to 8 nuclei which may be plasmacellular in type, but each nucleus contains a moderate-sized, dark blue nucleolus. The cytoplasm may contain less basophilic spongioplasm than the cell of Fig. 55 and a few scattered azurophil granules may be found within the central cytoplasmic area. In Fig. 57 the pattern of the two nuclei resembles that of a reticulum cell with prominent basophilic nucleoli, and the cytoplasm is composed of diffusely granular basophilic spongioplasm. Further illustrations of giant cells of this group are to be found in Rohr's[10] monograph (his Fig. 116) and in a recent work by Erf and Herbut,[45] who further attest to the value of the dry-fixed preparations as an adjunct to tissue sections in cytologic diagnosis.

Origin: Multinucleated myeloma cells may arise from the myeloblast (Fleischhacker and Klima[46]), and from the lymphocyte (Ewing[35]) or from the reticulum cell (Rohr[10]). Recently, Herbut and Erf[47] have described megakaryocytoid and lipoblastic giant myeloma cells in cases of multiple myeloma.

3. Giant Cells of Plasma Cell Leukemia.—Plasma cell leukemia is generally regarded as closely related to multiple myeloma (Lubarsch,[48] Moss and Ackerman,[49] Patek and Castle,[50] Jackson and associates[51]). Giant cells are less frequent than in multiple myeloma and, when they occur, rarely possess more than two nuclei (our Fig. 58). Such a cell from a dry-fixed bone marrow aspirate is depicted in Fig. 58. In structure it tends to resemble ordinary reactive multinucleated plasma cells more closely than the comparable myeloma cell. Patek and Castle[50] using the same technique depict a similar cell (their Fig. 2) and Osgood and Hunter[52] portray in colors (their Fig. 8) just such a cell with one of the nuclei very large and irregular and the cytoplasm containing azurophil granules.

H. Giant Cells in Systemic Disturbances Involving Lipids.—

1. Giant Cells in Niemann-Pick's Disease.—The giant cells in Niemann-Pick's disease are relatively rare, the mononuclear type predominating. According to Pick[53] these cells "include one or two small nuclei, occasionally many." The central cell of our Fig. 59, as seen in tissue section, contains three such nuclei. The nuclei are round or oval, peripheral and discrete, and the chromatin particles are small but numerous, so that the pattern is similar to that of the nucleus of a reticulum cell or a histiocyte. The abundant cytoplasm con-

PLATE XII

Fig. 55.—Multinucleated plasma cell in sternal bone marrow aspirate. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 97882.

Fig. 56.—Binucleated myeloma cell in a section of tumor in multiple myeloma. Hematoxylin and eosin stain; ×2000. AIP Neg. 98131.

Fig. 57.—Binucleated myeloma cell in a bone marrow aspirate in multiple myeloma. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 98132.

Fig. 58.—Binucleated plasma cell in sternal bone marrow smear in plasma cell leukemia. Dr. R. P. Custer's case. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 98129.

Fig. 59.—Multinucleated Niemann-Pick cells in a section of spleen. Hematoxylin and eosin stain; ×2000. AIP Neg. 97888.

Fig. 60.—Multinucleated Gaucher's cell in sternal bone marrow aspirate. Dr. R. P. Custer's case. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 97881.

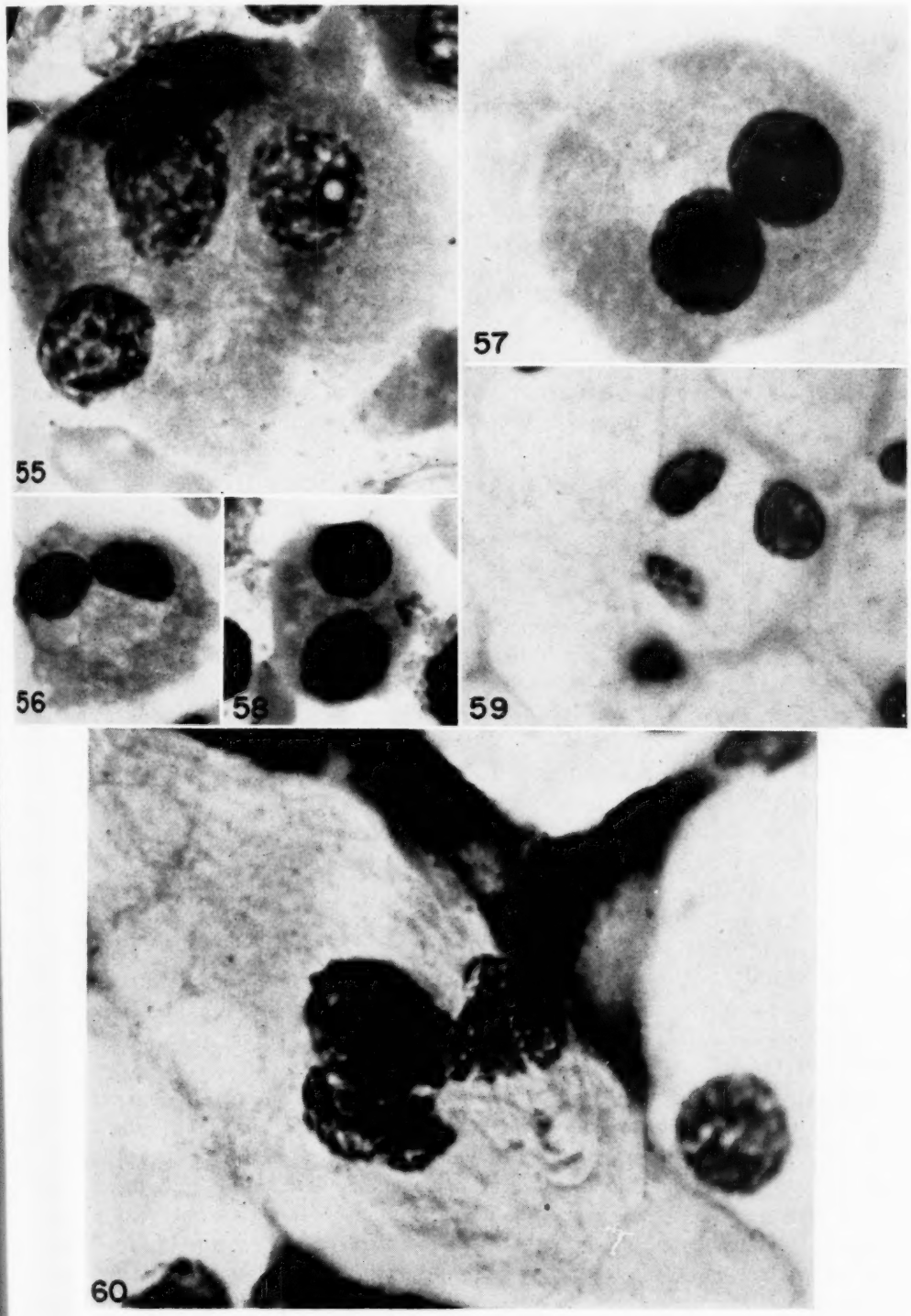


Plate XII

(For legend, see opposite page.)

sists merely of small and large, round vacuoles with thin intervening septa. The vacuoles are clear with ordinary staining methods, stain poorly (depending on mordant) with Nile blue and sudan III, but stain with the Smith-Dietrich method. The cells contain a phosphatide, sphingomyelin (Klenk[54]). For further cytologic detail and the histogenesis of this cell type the reader is referred to Bloom's[55] detailed monograph. The characteristics of these cells in dry-fixed preparations are well illustrated in photomicrographs of Pick[53] (his Fig. 1), in which the cells from the spleen contain a dense homogeneous central cytoplasm with the typical vacuoles peripherally situated, and of Rowland[56] (his Figs. 47 and 48), in which cells from the spleen and bone marrow show a more diffuse distribution of the vacuoles.

2. Giant Cells in Gaucher's Disease.—Giant cells are rather frequently found in Gaucher's disease although the mononuclear type still predominates. Such Gaucher cells are multinucleated. As seen in tissue sections, the discrete nuclei have reticulum cell or histiocytic characteristics although the chromatin content is more conspicuous. The cytoplasm stains poorly with sudan III, Smith-Dietrich, or Nile blue; however, Mallory's aniline blue, connective tissue stain reveals a cytoplasm filled with a dark blue fibrillar substance, weblike in arrangement, in contrast to the honeycombed or foamlike appearance of other lipoid-containing cells. In fact, the cytoplasm of Gaucher cells is frequently spoken of as being wrinkled. Piney and Hamilton-Paterson[41] state that the fibrils of these cells may be brought out by silver impregnation. The appearance of these cells in tissue sections is well portrayed by Mandelbaum and Downey.[57] Most authors state that the cells contain the cerebroside, cerasin, originally described by Lieb.[58]

The appearance of multinucleated Gaucher cells in dry-fixed preparations is similarly characteristic. The cells measure more than 50 microns in diameter. The oval or round discrete nuclei are relatively small and resemble reticulum cell nuclei, save that the irregular angular pieces of chromatin are coarser, although the chromatin-parachromatin distinction is preserved. A single, small, round basophilic nucleolus is present in each nucleus. Basophilic fibrils are to be found embedded in the colorless or faintly basophilic cytoplasm (our Fig. 60). As degeneration sets in, however, vacuoles may also appear in the cytoplasm of these cells.

3. Giant Cells in Hand-Schüller-Christian Disease.—The majority of the giant cells seen in the xanthomatous lesions of this disease are lipid-containing but otherwise ordinary foreign body giant cells. The cytoplasm stains well with sudan III. The cells contain cholesterol and cholesterol esters as described originally by Pinkus and Pick[59] in symptomatic xanthomatoses. A more spectacular type of giant cell called the Touton giant cell[60] (Fig. 61) is occasionally found. This cell presents a rim of closely spaced discrete nuclei of the histiocytic type about an area of dense, homogenous, acidophilic cytoplasm. The ring of nuclei in turn is surrounded by an abundant, peripheral, foamy cytoplasm. It must be emphasized that cells of this type are not specific for Hand-Schüller-Christian disease but may be found in the so-called secondary xanthomatoses as well; in fact, the cell depicted in Fig. 61 was found in an area of fatty degeneration within a kidney, the seat of chronic pyelonephritis. The structure

of the so-called nonspecific lipoid histiocytes is apparently not well known; Paseyro[21] (his Fig. 113) depicts such a large vacuolated cell from the bone marrow in a case of diabetic xanthomatosis. For further interesting details the reader is referred to the monograph of Thannhauser and Magendantz[61] upon this subject.

4. Normal Fat Cells.—Normal fat cells within the blood-forming organs present few structural features that are of differential significance as seen in tissue sections. However, in dry-fixed preparations these large cells become flattened and distorted; they measure more than 60 microns in diameter and are usually round with an eccentric nucleus of the histiocytic type and an abundant colorless cytoplasm. As the cell body is broken up the cytoplasm takes on a wrinkled appearance and a faint basophilic tinge; at the same time numerous small vacuoles appear, particularly about the nucleus (our Fig. 62).

5. Nonspecific, Fat-Containing Foreign Body Types of Giant Cells.—These cells are not uncommonly found in the blood-forming organs, particularly the lymph nodes, under many and diversified conditions (Fig. 63). They are frequently seen in or about reactive or degenerative processes.

I. Giant Cells in Measles (Warthin-Finkeldey Giant Cells).—These giant cells are variously found in the germinal centers and surrounding lymphoid tissue of the abdominal lymph nodes, spleen, tonsils, appendix, or lymphoid tissues of the respiratory and gastrointestinal tracts, usually in the prodromal period of measles. Giant cells were first observed in measles by Alagna[62] who believed they somewhat resembled megakaryocytes. Warthin[63] and Finkeldey[64, 65] gave the first accurate descriptions of this specific giant cell type. They are large syncytial multinucleated giant cells containing from 5 to 50 or more nuclei. The overlapping nuclei are generally arranged in a grape-like or mulberry-shaped cluster bordered by a scant rim of eosinophilic cytoplasm. In the germinal and reaction centers the nuclei tend to be of the pale, vesicular, reticulum cell type (Fig. 64), with several small- or moderate-sized nucleoli. In the surrounding lymphoid tissue are numerous similar cells (Fig. 65) with nuclei containing coarser chromatin particles resembling plasma cell nuclei. The nuclei in this latter cell type are somewhat more diffusely arranged, but the cytoplasm remains as a scant acidophilic border. Warthin[63] found no intracellular microorganisms or inclusions within the cytoplasm of these cells, but Hathaway[66] described them as being phagocytic.

Origin: Warthin[63] regarded these giant cells as derived from cells of the lymphoblast type. Mulligan[67] concluded that they arose by a "polynuclear abnormal development of the stem cell." The consensus is that they arise by amitotic division of reticulum cells, mesenchymal cells, plasma cells, or lymphocytes or by fusion of lymphocytes (Corbett[68]). In Fig. 66 is depicted the origin of such a cell in a distorted, reactive, multinucleated reticulum cell.

J. Foreign Body Types of Giant Cells (Langhans' Giant Cell).—In 1868 Langhans[69] described the cell in tuberculosis which now bears his name. It is a multinucleated giant cell having from 2 to more than 1,000 round or oval nuclei. In tissue sections the nuclei contain fine chromatin particles and a small

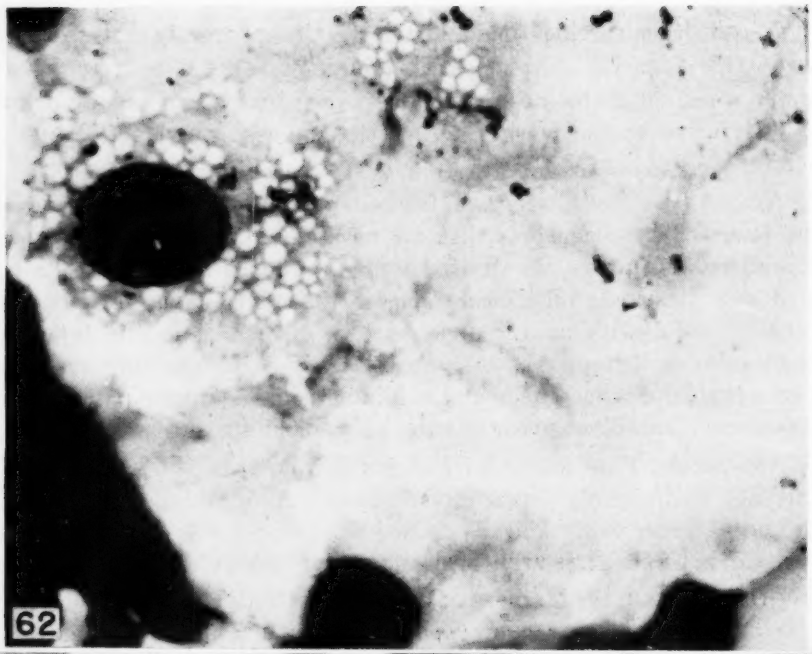


PLATE XIII

Fig. 61.—Touton giant cell in section of kidney with fatty degeneration in chronic pyelonephritis. Hematoxylin and eosin stain; ×2000. AIP Neg. 98138.

Fig. 62.—Distorted fat cells in sternal bone marrow aspirate. May-Grünwald-Giemsa stain; ×2000. AIP Neg. 97883.

Fig. 63.—Fat-containing foreign body giant cell in section of reactive omental lymph node. Hematoxylin and eosin stain; ×2000. AIP Neg. 97001.

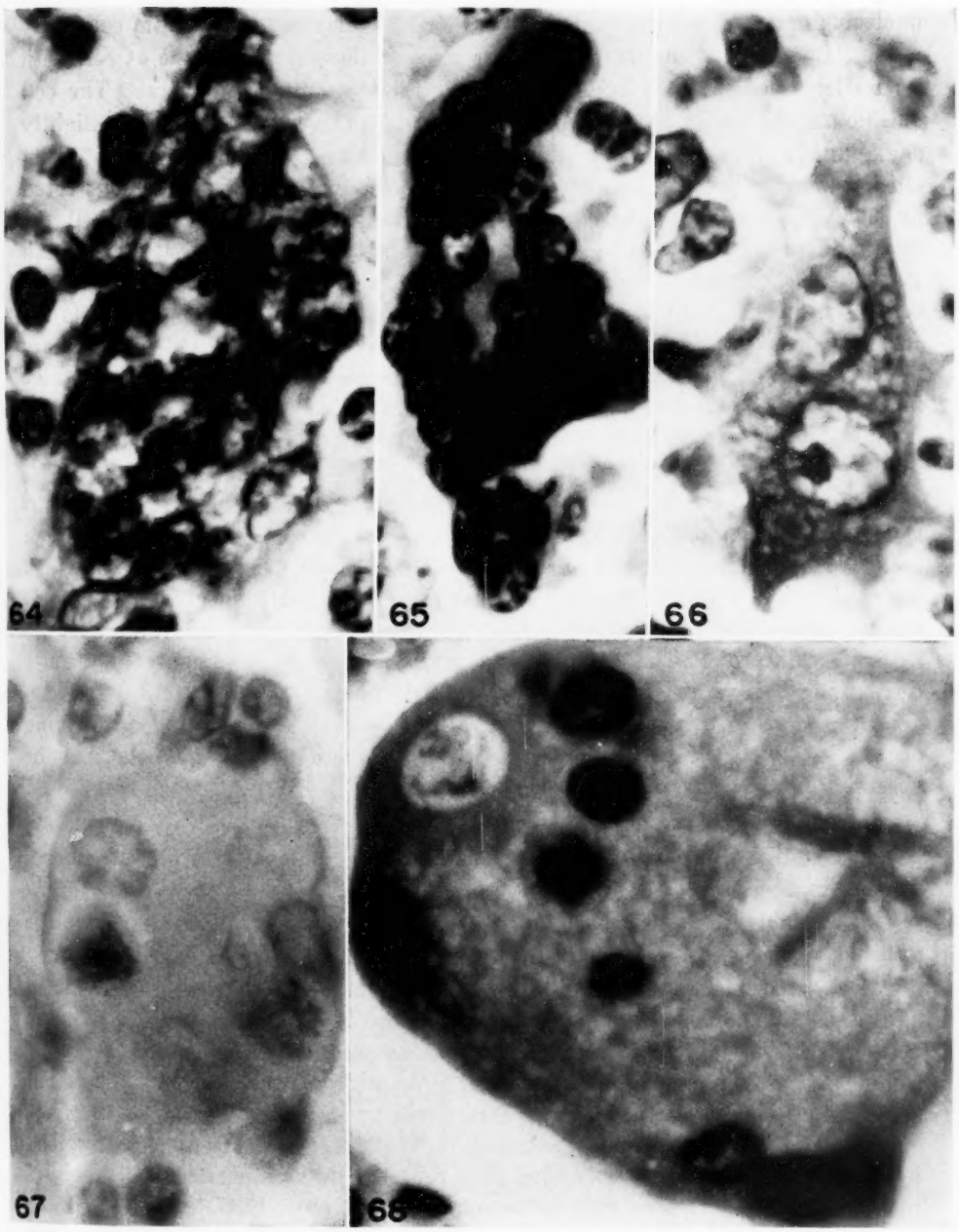


PLATE XIV

Fig. 64.—Warthin-Finkeldey giant cell in section of lymphoid tissue of appendix in prodromal stage of measles. Hematoxylin and eosin stain; ×2000. AIP Neg. 96999.

Fig. 65.—Warthin-Finkeldey giant cell in section of lymphoid tissue of appendix in prodromal stage of measles. Hematoxylin and eosin stain; ×2000. AIP Neg. 96994.

Fig. 66.—Atypical enlarged reticulum cell in section of lympoid tissue of appendix in prodromal stage of measles. Hematoxylin and eosin stain; ×2000. AIP Neg. 96997.

Fig. 67.—Foreign body giant cell containing masses of *mycobacterium leprae* in section of lymph node in leprosy. Kinyoun's stain; ×2000. AIP Neg. 97252.

Fig. 68.—Foreign body giant cell containing asteroid in section of lymph node in leprosy. Hematoxylin and eosin stain; ×2000. AIP Neg. 97250.

nucleolus or two; they resemble the nuclei of histiocytes or reticulum cells. By usage "Langhans' type giant cell" has come to designate the type of cell with its nuclei arranged in a ring near the periphery of the cytoplasm. The cell outline may be round, oval, irregular, or stellate. The cytoplasm itself is slightly acidophilic and may be finely granular or homogeneous. At times ingested inclusions may be present, that is *Mycobacterium tuberculosis* may be demonstrated by acid-fast methods. Actually, however, Langhans not only described the cells with the peripheral ring of nuclei but also those with nuclei at one or both poles of the cell body, scattered diffusely throughout its substance or placed centrally. This group of cells without the ring of nuclei is referred to as the foreign body type of giant cells by modern writers. Actually both groups are nonspecific. In infectious granulomas the so-called Langhans' type may be more frequently observed, whereas the foreign body type of nuclear arrangement is more likely to be found about inert foreign substances, although this is by no means invariable.

Foreign body giant cells arise by fusion of individual macrophages (histiocytes) or by amitotic division of their nuclei without constriction of the cell bodies. The macrophages in turn arise from lymphocytes and monocytes which have migrated from the blood or from cells of the fixed connective tissues possessing phagocytic abilities. The blood-forming organs, abounding as they do in fixed connective tissue cells with the ability of becoming phagocytic, usually draw upon this source for production of their phagocytic giant cells. It is beyond the scope of this review to include all of the affections of the blood-forming organs in which the presence of the various foreign body giant cells may be observed; however, some of them are herein listed:

1. Tuberculosis (Langhans[69]).

2. Syphilis, especially in the secondary stage (Michelson[70]), but not confined to that stage.

3. Actinomycosis (Forbus[71] in his Fig. 349 depicts a rod-shaped sulfur granule of *Actinomyces bovis* being ingested by a foreign body giant cell).

4. Leprosy. Langhans' and foreign body giant cells may be present in affected lymph nodes (our Figs. 67 and 68). With acid-fast techniques some of these cells are found to contain large numbers of *Mycobacterium leprae* arranged as packets and globi (Fig. 67). An additional finding, although not specific for leprosy, is the peculiar structures occasionally observed within the cytoplasm of such cells, called "asteroids" (Fig. 68). These were first described by Wolbach.[72] They are embedded in a round, pale staining area of the cytoplasm and consist of a central dense zone from which radiate from 10 to 15 or more sharp or blunt spindles, the entire structure being more or less star shaped. Asteroids are usually acidophilic and have been observed in the giant cells of tuberculosis, Boeck's sarcoid, and histoplasmosis as well as of leprosy, to mention but a few.

5. Lymphogranuloma venereum (lymphopathia venereum). In Fig. 69 is depicted a giant cell of the Langhans' type in the zone of palisaded histiocytes about a typical stellate abscess in an inguinal lymph node in this disease (Ash and Spitz[73]).

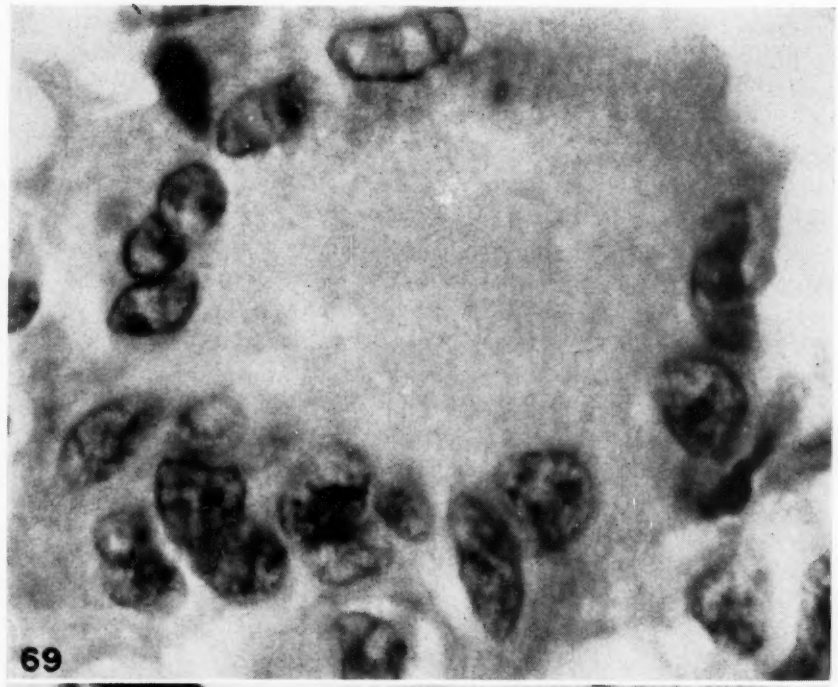


PLATE XV

Fig. 69.—Langhans' type giant cell in section of inguinal lymph node in lymphogranuloma venereum (lymphopathia venereum). Hematoxylin and eosin stain; ×2000. AIP Neg. 97620.

Fig. 70.—A portion of a large foreign body giant cell containing a Schaumann body in section of lymph node in Boeck's sarcoid. Hematoxylin and eosin stain; ×2000. AIP Neg. 97619.

6. Visceral leishmaniasis, Kala-azar (Meleney[74]).

7. Boeck's sarcoid (Schaumann[75]). Foreign body giant cells are frequently observed in the granulomatous lesions of this disease. Rare cells may contain a large, laminated, deeply basophilic, roughly spherical structure called the "Schaumann body" (Fig. 70). These bodies are rather specific for Boeck's sarcoid although similar structures are occasionally to be found in other conditions.

8. Coccidioidomycosis. Shown in Fig. 71 is a giant cell which contains the causative organism, *Coccidioides immitis,* a nonbudding cell form undergoing endosporulation. Note the thick, double-contoured capsule.

9. Paracoccidioidal granuloma, South American blastomycosis (Moore[76]).

10. Histoplasmosis. In Fig. 72 is depicted an irregular type of foreign body giant cell in which numerous organisms are to be found. *Histoplasma capsulatum* appears as a very small yeastlike cell with a prominent nucleus and a thick refractile capsule. The small portion of free cytoplasm immediately above the giant cell in Fig. 72 likewise contains several such organisms.

As mentioned in the section on the giant cells in Hodgkin's disease, it should be kept in mind that nonspecific and merely reactive foreign body giant cells may occur in lymphomas and related affections of the reticulo-endothelial system.

For further details of the morphology and occurrence of foreign body giant cells in specific diseases the reader is referred to the excellent monographs of Haythorn[1] and Ash and Spitz.[73]

K. Miscellaneous Giant Cells and Structures Resembling Giant Cells.—

1. Nonspecific Reactive Giant Cells.—Frequently in the nonspecific subacute and chronic inflammations affecting particularly the lymph nodes and spleen, a few reactive forms of giant cells are found of which little previous mention has been made in the literature. They consist of small foreign body giant cells with a few irregularly situated histiocytic nuclei, or they are polylobular mononuclear forms (Fig. 73), probably representing a reactive multilobulation of the reticulum cell nucleus. Their nuclei have small basophilic nucleoli and varying amounts of granular chromatin. The cell outline is usually irregular and the faintly acidophilic cytoplasm is not remarkable. Occasionally many fat-con-

PLATE XVI

Fig. 71.—A portion of a large foreign body giant cell containing *Coccidioides immitis* in section of lymph node in coccidioidomycosis. Hematoxylin and eosin stain; ×2000. AIP Neg. 97248.

Fig. 72.—Foreign body giant cell and detached cytoplasm containing Histoplasma capsulatum in section of lymph node in histoplasmosis. Hematoxylin and eosin stain; ×2000. AIP Neg. 97886.

Fig. 73.—Atypical enlarged reticulum cell in section of reactive lymph node. Hematoxylin and eosin stain; ×2000. AIP Neg. 97007.

Fig. 74.—Giant cell in section of lymph node in brucellosis. Hematoxylin and eosin stain; ×2000. AIP Neg. 98135.

Fig. 75.—Giant cell in section of lymph node in disseminated lupus erythematosus. Hematoxylin and eosin stain; ×2000. AIP Neg. 98130.

Fig. 76.—Phagocytic histiocyte in section of spleen in idiopathic pulmonary fibrosis. Hematoxylin and eosin stain; ×2000. AIP Neg. 97011.

Fig. 77.—Modified capillary in section of lymph node. Hematoxylin and eosin stain; ×2000. AIP Neg. 97617.

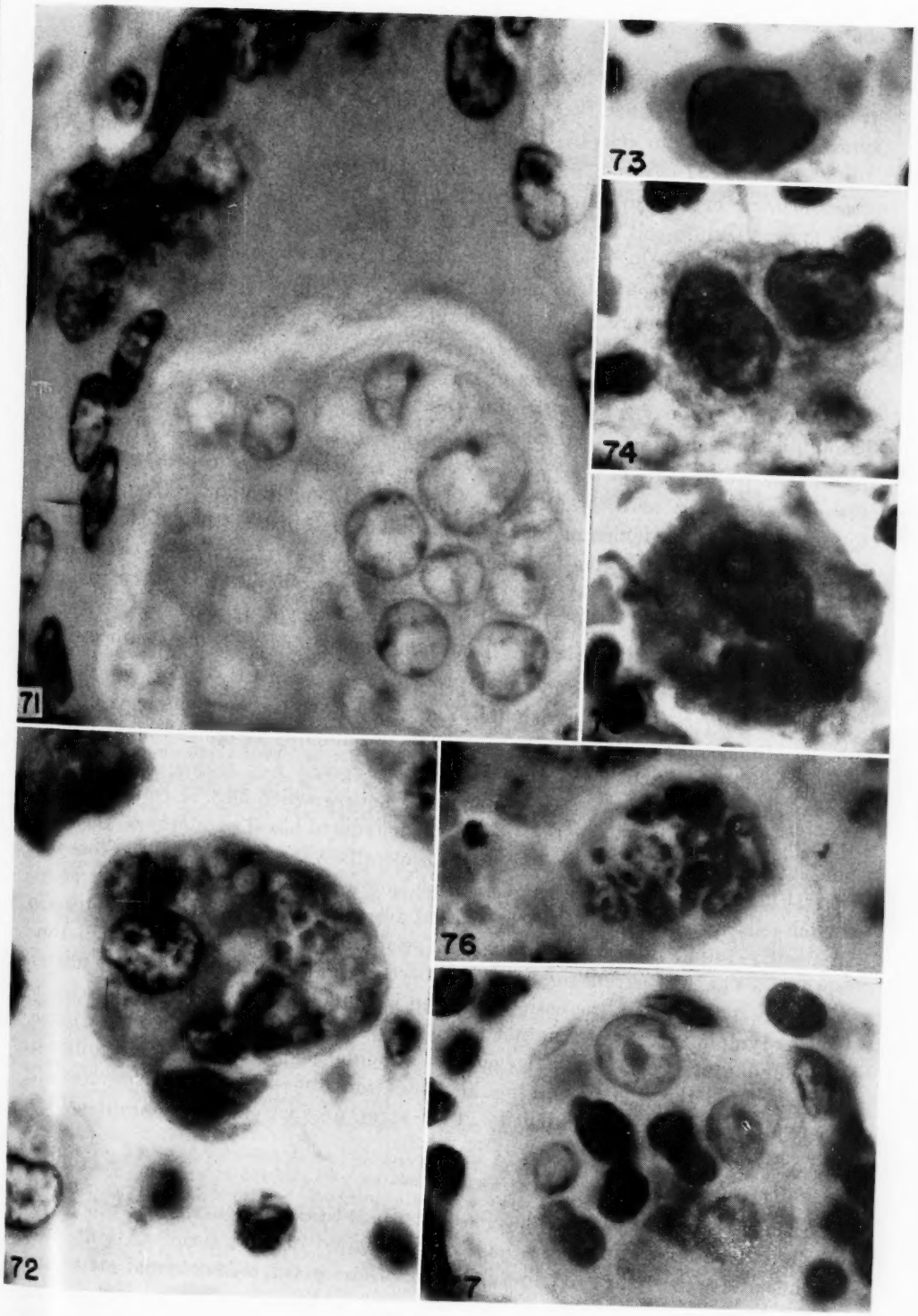


Plate XVI

(For legend, see opposite page.)

taining vacuoles are to be found within the cell body (Fig. 63). These types are of importance because they have at times been confused with megakaryocytes or with the giant cells of Hodgkin's disease and reticulum cell sarcoma.

2. The Giant Cells in Brucellosis.—In certain relatively rare reactions of the blood-forming organs to brucella, particularly *Brucella melitensis,* the histiocytic and reticulum cell reaction may present nonspecific pleomorphism similar to that described in the preceding paragraph. Represented in Fig. 74 is just such a type of giant cell in the lymph node from a case of brucellosis proved by both blood cultures and recovery of the organism after animal inoculation. These cells have been spoken of as simulating the cells of Hodgkin's disease. However it is apparent that the nuclei of the giant cells of brucellosis are like those of nonspecific histiocytes or reticulum cells, with small although circumscribed basophilic nucleoli.

3. The Giant Cells in the Lymph Nodes of Disseminated Lupus Erythematosus.—Recently Fox and Rosahn[17] have described a type of giant cell frequently found in the lymph nodes in cases of disseminated lupus erythematosus. It is superficially somewhat like a megakaryocyte, possessing one or two large multilobular nuclei with a greater chromatin content than the customary reticulum cell nucleus (Fig. 75). Unlike a megakaryocyte, however, it lacks evidence of pseudopodial platelet formation, its cytoplasm being composed of a homogeneous, pale, or acidophilic substance.

4. Scavenger Cells.—Occasionally large phagocytic mononuclear macrophages which have ingested numerous nuclear remnants (Fig. 76) may be mistaken for unusual giant cell forms.

5. Capillary Cross Section.—Another structure which may be confused with abnormal giant cell types is the specially constructed blood capillary of the lymphatic tissue of the lymph nodes and the appendix when it is viewed in cross section (Fig. 77). These capillaries are lined by a single layer of cuboidal endothelial cells instead of those of the usual pavement cell type. Lymphocytes are frequently found migrating through the walls of these structures, which thus serve as a mode of egress for the lymphocytes in addition to the efferent ducts. When, as is frequently the case, the lumen is found to contain red corpuscles as well as lymphocytes, the true nature of the structure is apparent at a glance. In inflammatory reactions, however, the cuboidal cells may become swollen so as to occlude the lumen, making identification difficult unless serial sections are available; in these the identity of the structures is readily apparent (Maximow and Bloom[18]).

COMMENT

The structure of the giant cells of the blood-forming organs in both physiologic and pathologic processes has been presented in atlas form. Attention of the hematologist is directed to variations of the giant mesenchymal structures of the hematopoietic organs in conditions not ordinarily of prime hematologic interest, for the reason that such affections are of the same stromal site or origin as are many of the blood dyscrasias. The use of dry-fixed smears or impression

preparations of the blood-forming organs in conjunction with tissue sections affords another source of information regarding structural characteristics of many giant cell types.

It is hoped that the collection of photomicrographic representations of the many giant cell types within the scope of a single article will provide an opportunity for ready comparison of both related and unrelated giant cell forms and will serve as an aid in the determination of the nature and degree of such relationships or differences.

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MITOCHONDRIA AND THEIR RELATION TO THE SO-CALLED HYALOPLASM

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INTRODUCTION

DURING the past fifteen years considerable morphologic evidence has been collected which indicates that genuine megaloblasts have no role in normal erythropoiesis.[1, 2] But megaloblasts do, however, play the major role in the pathologic erythropoiesis which occurs in bone marrow from patients with a liver principle deficiency anemia. Before arriving at these conclusions, it was necessary to study material similar to that which Doan and associates[3] used while formulating their theory for avian and mammalian erythropoiesis. Because of the studies of Sabin[4] in 1921 on vitally stainable granulation in erythroblasts, blood drawn from chick blastoderms on the second and third days of incubation was considered the best single source of cells which Doan and associates would accept unequivocally as megaloblasts. In my studies of the blood from these chick blastoderms it was considered desirable not only to study it vitally according to Sabin's method, but also to employ Doan and associates' supravital technique and to check Sugiyama's[5] findings with Wright's stain.

Although my observations on chick erythroblasts stained vitally with neutral red confirm those made by Sabin, observations made on Wright stained dry films did not correspond to either Sabin's or Sugiyama's description. First, the nuclei were not as empty or devoid of structure as described by Doan and associates, nor as poorly preserved as illustrated by Sugiyama. Second, the cytoplasm of these primitive red blood cells had much more structure than described by Sabin and it differed in degree from the cytoplasm described and illustrated by Sugiyama. The thing which attracted my attention was the presence in the cytoplasm of pale or yellowish areas of hyaloplasm, sometimes discrete, not unlike those described by Downey[6] in cells from certain cases of leukemic reticulo-endotheliosis. These observations alone did not seem to be so important until similar cells stained supravitally with either janus green or pinacyanol were found to have a distribution of mitochondria practically identical with the areas of hyaloplasm as seen in dry fixed smears. Because of the apparent high degree of correlation between these structures seen in cells prepared by two different methods, it was tentatively concluded that the so-called hyaloplasm represented the negative images of mitochondria. Due to an interest in the placental transfer of antipernicious anemia principle[7] and the development of a presumptive test for it,[8] studies made originally on chick primitive erythroblasts were carried over to similar cells in the yolk sac of the eleven-day rat embryo. Normally these yellowish areas or flecks in the basophilic cytoplasm are distributed so as to fall into three main categories.

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Research was aided by the Dr. Grant T. Fisher Fund.

namely, diffuse, perinuclear, and a juxtanuclear clump. The latter was of particular interest since the administration of liver extract, desiccated hogs' stomach, or synthetic folic acid produced an increase in the number of cells having a juxtanuclear clump of the cytoplasmic pale areas in dry fixed material and of the mitochondria in the supravital material.

The present paper describes results which represent the termination of an extended period of observation and the beginning of an analysis of the cytoplasm with various techniques applicable to the material studied.

METHODS

Twenty dozen chicken eggs were incubated for periods ranging from two to nine days. Blood was drawn from the blastoderms and studied according to the techniques described by Sabin[4] and Sugiyama.[5] Pinacyanol[9] was usually the dye use for supravital technique, but janus green and neutral red were also used, especially for the studies on chick blood cells.[10] Throughout the course of investigating several related problems, 4,187 eleven-day rat embryos were obtainted from seventy-nine control and 308 test pregnant Wistar rats. Blood from the yolk sac[11] was studied by means of various techniques. In supravital studies, amniotic fluid was withdrawn by means of a fine capillary pipette before delivering the rat yolk sac. A drop of this was placed on a specially prepared slide and yolk sac swished around in it. Then the preparation was covered and sealed with petroleum jelly. Although blood cells from embryos whose mothers received various antianemic substances were studied as the occasion arose, only observations on normal yolk sac blood are considered in this article. Dry films of blood from 1,657 embryos were stained with the May-Grünwald-Giemsa combination of Pappenheim within six hours after they were made. Permanent mitochondrial preparations were made from dry films of both embryonic chick and rat blood cells. The former were fixed and stained according to Schridde's[12] original technique, with the exception that differentiation was carried out in 1 per cent methyl green rather than picric acid alcohol. Practically all of the mitochondrial preparations of embryonic rat blood cells were made by adapting the standard Bensley-Cowdry technique[13] to this material. Cover slip preparations were dried rapidly and placed immediately in either Regaud's formalin bichromate or acetic osmic bichromate for eighteen to twenty-four hours. They were washed in tap water for two or three minutes followed by a brief bath in distilled water. The use of permanganate and oxalic acid was unnecessary. The cover slip preparations with the fixed film directed upwards were placed on slides to facilitate handling while staining with aniline acid fuchsin. Heat was applied for ten seconds and the preparation allowed to cool. This was repeated two or three times. Either methyl green or Wright's stain applied for ten to fifteen seconds was used for differentiation and counterstaining. Dehydration was accomplished by rapidly passing the cover slips through two changes of absolute alcohol followed by xylene. The distribution and localization of lipoid material was studied with the aid of sudan black, which was introduced by Lison[14] and later employed by Baker[15] for the study of Golgi element. Freshly dried cover slip preparations were placed in Baker's formol-calcium fixative for eighteen to twenty-four hours. After washing in distilled water for one or two minutes, they were passed through 50 and 70 per cent alcohol and stained in a saturated solution of sudan black in 70 per cent alcohol for ten minutes. Cover slips were placed with the fixed smears facing downward in the sudan black in order to prevent dye particles from collecting on cells. Small glass rods or a metal paper clip were used to elevate the cover slips from the bottom of the staining dish and thereby permit an even distribution of stain. Excessive amounts of the dye were extracted by passing the preparation through three changes of 50 per cent alcohol for exactly ten seconds in each change of alcohol. They were then washed in distilled water for about two minutes and mounted in glychrogel[16] No counter stain was used. The presence of vitamin A was detected by using the Carr-Price reagent of 30 per cent antimony trichloride in chloroform.[17] Cover slip preparations, which had been kept in the dark, were inverted on a clean slide and two parallel

edges were sealed with petroleum jelly. This aided in channeling the reagent and prevented the cover slip from moving when the immersion lens was focused. The preparation was then examined under low power for a suitable field of primitive erythroblasts which when found was examined with the oil immersion lens. The reagent was permitted to flow beneath the cover slip by capillary attraction. The presence of vitamin A was indicated by a fugitive light greenish blue color reaction.

Phase microscopy[18] has been a great aid in this problem. The optical differentiation of cytoplasmic structures having small differences in their optical path was made possible by selecting an oil immersion lens with the proper diffraction plate. Bright contrast was obtained with a 1.8 mm. objective, 0.2A + 0.25 λ, and dark contrast with 1.8 mm. objectives, 1.0B–0.25 λ and 2.3B–0.33 λ. Unstained dry films were inverted on a drop or two of either Baker's formol-calcium or 10 per cent formalin and ringed with petroleum jelly. Such preparations have remained in good condition for two months. After studying cells with phase microscopy, it was then possible to mark them for restudy with a bright field microscope following Wright's stain or Baker's formol-sudan black. Oil and petroleum jelly were removed by camel's hair brush moistened with xylene. Cover slip preparations were washed briefly in distilled water, dried, and stained with Wright's stain. They were again washed briefly in distilled water, dried in air, cleared in xylene, and mounted in clarite. Both bright- and dark-contrast phase microscopy were used with sudan black preparations. Although dark contrast was used in combination with the supravital technique, preference was given to studies of unstained dry films with bright contrast phase microscopy. The combination of dark contrast phase microscopy and sudan black brought out more detail than either technique alone. All of these studies were done with a Spencer research microscope.

OBSERVATIONS

A. Embryonic Chick Blood Cells.—

1. Dry Films of Blood From Chick Blastoderms Incubated Two Days: This material should be of interest to all students of the megaloblast-normoblast problem since, according to Doan and associates[3] and Sabin,[4] practically all of the cells present are megaloblasts, in the sense of their definition. However, no matter how intriguing this phase of the problem may be, it must not detract from our present interest in the cytoplasm. Some of these cells are illustrated on Plate I and designated as primitive erythroblasts according to Maximow.[19] Regardless of whether Wright's stain or May-Grünwald-Giemsa is used, one of the most striking characteristics is the very basophilic cytoplasm which agrees with the observations made by Doan and associates[3] and Dawson[20] but not with those of Sugiyama[5] who described it as having a reddish tinge. Another constant characteristic of this basophilic cytoplasm is that it is not uniform and homogeneous, contrary to Doan and associates[3] universal description for such cells. It is mottled with light areas of what Pappenheim[21] called hyaloplasm (paraplasm). These areas are neither uniform in their size and shape, nor their distribution. For example, in cell *1, a*, Plate I, there are faint filamentous light areas, while in cell *1, b*, there is a definite juxtanuclear light area in addition to others scattered throughout the cytoplasm. The juxtanuclear area of cell *1, b*, may be what some authors have called the region of the centrosphere, which according to Dawson[20] is indistinct but according to Sugiyama[5] appears as a clear red spot. At no time were large perfectly clear spots lacking substance found as described and illustrated by Sugiyama. Such an area in his Fig. 4 was in all probability an artifact.

The nuclei of these primitive erythroblasts have one or two large well defined nucleoli. They may be round or irregular. The nuclear chromatin and its characteristic pattern, however, are something which escaped the attention of Sabin,[4] Doan and associates,[3] Sugiyama,[5] and Dawson.[20] It is very definitely not pale, scanty, and obscure as these authors have reported. To the contrary, the nuclear pattern is quite distinctive and is not identical with that of any cell normally found in the bone marrow. The chromatin is arranged in more or less angular particles varying in size. They are not as uniform in size and distribution as those of genuine megaloblasts of pernicious anemia bone marrow during relapse.[1, 2, 22] There is a sharp demarcation between the chromatin and parachromatin (basi- and oxychromatin). Although the parachromatin is conspicuous in some cells, it never exceeds the chromatin in amount. Some of the chromatin particles have delicate strands connecting them. There is usually a marked tendency for it to concentrate in the vicinity of the nucleoli. Sugiyama's[5] Fig. 8 does a great injustice to these cells.

2. Supravital Preparations of Blood From Chick Blastoderms Incubated Two Days: Either janus green or pinacyanol was used for these studies. In general, the mitochondria were either rod or coccoid forms like those described by Doan and associates[3] for the rabbit and Sugiyama[5] for the chick. Their number varied from 28 to 57. In addition to these there were also occasional filamentous forms which did not stain as intensely as the other forms. In no instance were the long filaments the only type of mitochondria present as reported by Dawson.[20] In many cells the mitochondria were scattered throughout the cytoplasm but in others they had a very definite perinuclear and juxtanuclear arrangement not unlike the clear areas shown in cell *1, b*, Plate I. Their position seemed to be relatively stable since they would maintain their perinuclear distribution even though the cells were flattened and made to move by gentle pressure on the cover slip. They really gave the impression of clinging to the nucleus as Cunningham and Tompkins[23] have described for other cells. In dead or damaged cells, the mitochondria would move and oscillate, indicating a change in the cytoplasm from a gel to sol state.

After preparations were studied with a bright-field microscope, they were then examined with dark-field illumination; the deep purple or dark blue mitochondria stained with pinacyanol appeared orange and the green mitochondria stained with janus green appeared reddish. However, the most important finding was that not all of the mitochondria had been stained selectively. The number of unstained refractile mitochondria varied from 1 to 6 per cell. No counts were made to determine the relative proportion of cells with all of their mitochondria stained. It was first thought that the concentration of the dye, its distribution on the slide, or the duration of exposure might have something to do with the nonstaining of some mitochondria, but subsequent studies on the rat primitive erythroblast have lead to the conclusion that mitochondria within a single cell differ in their composition.

3. Permanent Mitochondrial Preparations of Blood From Chick Blastoderms Incubated Two Days: After the previously stated observations were made it

was tentatively concluded that the light areas, as seen in dry films of primitive erythroblasts stained with the usual hematologic dyes, represented negative images of mitochondria. Investigations of this problem along more critical and analytical lines had to be postponed for about seven years. However, these observations have now been verified and another method of study was introduced. It was felt that since all mitochondria do not stain supravitally, and this reaction is probably due to the presence of a proteolytic enzyme,[24] one of the more specific techniques should be applied to this material. Only moderate success was obtained when Schridde's modification of Altmann's technique was used.[12] The cells were rounded up and the distribution of mitochondria was not readily made out. Then too, a great many cells were lost from the cover slips. Both of these difficulties were overcome when the first part of Freifeld's technique[25] was used, namely, the rapid drying of films or imprints before immersion in the fixing fluid. It is understood that this means drying in air at room temperature until the films appear dry. After examining these preparations and noting the clear-cut mitochondrial distribution as that in cells *2*, *a*,

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PLATE I

All cells were photographed at the same magnification of 1850 diameters. Cell *X* is a red blood cell from the maternal placental circulation to be used as a comparison of size. Nuclei of most cells are out of focus in order to bring out cytoplasmic detail. With the exception of cells *1* and *2*, all cells are primitive erythroblasts from the yolk sacs of eleven-day rat embryos.

1, Blood from chick blastoderm after two and one-half days incubation. Wright's stain. Cytoplasm is quite basophilic. Light areas of the so-called hyaloplasm vary in shape and distribution. Cell *1*, *a*, has filamentous clear areas; cell *1*, *b*, a juxtanuclear clump; and cell *1*, *c*, a diffuse distribution.

2, Blood from chick blastoderm after two and three-quarter days incubation. Modified Schridde's mitochondrial technique using methyl green for differentiation and counterstaining. Most of the mitochondria tend to be cocci shaped but some short plump rods and filaments were present. In cell *2*, *a*, the mitochondria have a diffuse distribution. Faint filaments are present in the lower portion of the cytoplasm. Cell *2*, *b*, has a perinuclear distribution of mitochondria.

3, May-Grünwald-Giemsa stain. Cytoplasm is deeply basophilic. This cell is of particular interest because there are filamentous areas of the so-called hyaloplasm. Some of these areas are quite curved and may appear horseshoe shaped like that shown in the lower left portion of the cytoplasm.

4, May-Grünwald-Giemsa stain. Cytoplasm is quite basophilic with a more or less diffuse arrangement of yellowish areas of the so-called spherical area in the upper left portion of the cytoplasm. See text for discussion of its probable nature.

5, May-Grünwald-Giemsa stain. Most of the cytoplasm is very basophilic and fairly homogeneous. Note that the light areas of so-called hyaloplasm have a perinuclear arrangement. The small, dark rodlike structure at the upper margin of the cell is a piece of dust.

6, May-Grünwald-Giemsa stain. The nonmitotic cell *6*, *a*, has a juxtanuclear clump of the so-called hyaloplasm. Some authors would describe this as a Hof in the region of the cytocentrum. Careful focusing revealed that clear areas of cells *5* and *6*, *a*, are not homogeneous but contain many closely packed discrete yellowish or pale areas. Cell *6*, *b*, is in prophase of mitosis and has a deeply basophilic cytoplasm with a diffuse distribution of yellowish areas.

7, Bensley-Cowdry aniline acid fuchsin technique applied to dry smear. Most of the mitochondria are cocci shaped but there are some short rods. Note their diffuse distribution.

8, Bensley-Cowdry aniline acid fuchsin technique applied to dry smear. Morphology of mitochondria is the same as in cell 7, but the distribution is quite different. There is a good correlation between perinuclear clear areas as shown in cell *5* and the perinuclear arrangement of mitochondria.

9, Bensley-Cowdry aniline acid fuchsin technique applied to dry smear. Morphology of mitochondria is the same as in cell 7, but the distribution is different from either of the preceding cells. Although there is a juxtanuclear clump of mitochondria, there are still some scattered throughout the cytoplasm. Note the correlation between this type of mitochondrial distribution and the juxtanuclear position of the clear area in cell *6*.

10, Baker's formol-sudan black technique. The mitochondria appear as gray or grayish black cocci and short plump rods. Some filamentous forms may also be seen, but they for the most part do not take on as much sudan black as the other forms. This cell has a diffuse arrangement of mitochondria.

11, Baker's formol-sudan black technique. The mitochondria appear as described for cell *10*, but here they have a perinuclear distribution with some slightly out of focus superimposed on the nucleus.

12, Baker's formol-sudan black technique. Morphology of mitochondria is the same as in cell *10*, but the distribution is that of a juxtanuclear clump. Faint filaments are present in the lower portion of the cytoplasm and they have varying degrees of curvature. The two black spheres to the left of the nucleus belong to the Golgi element.

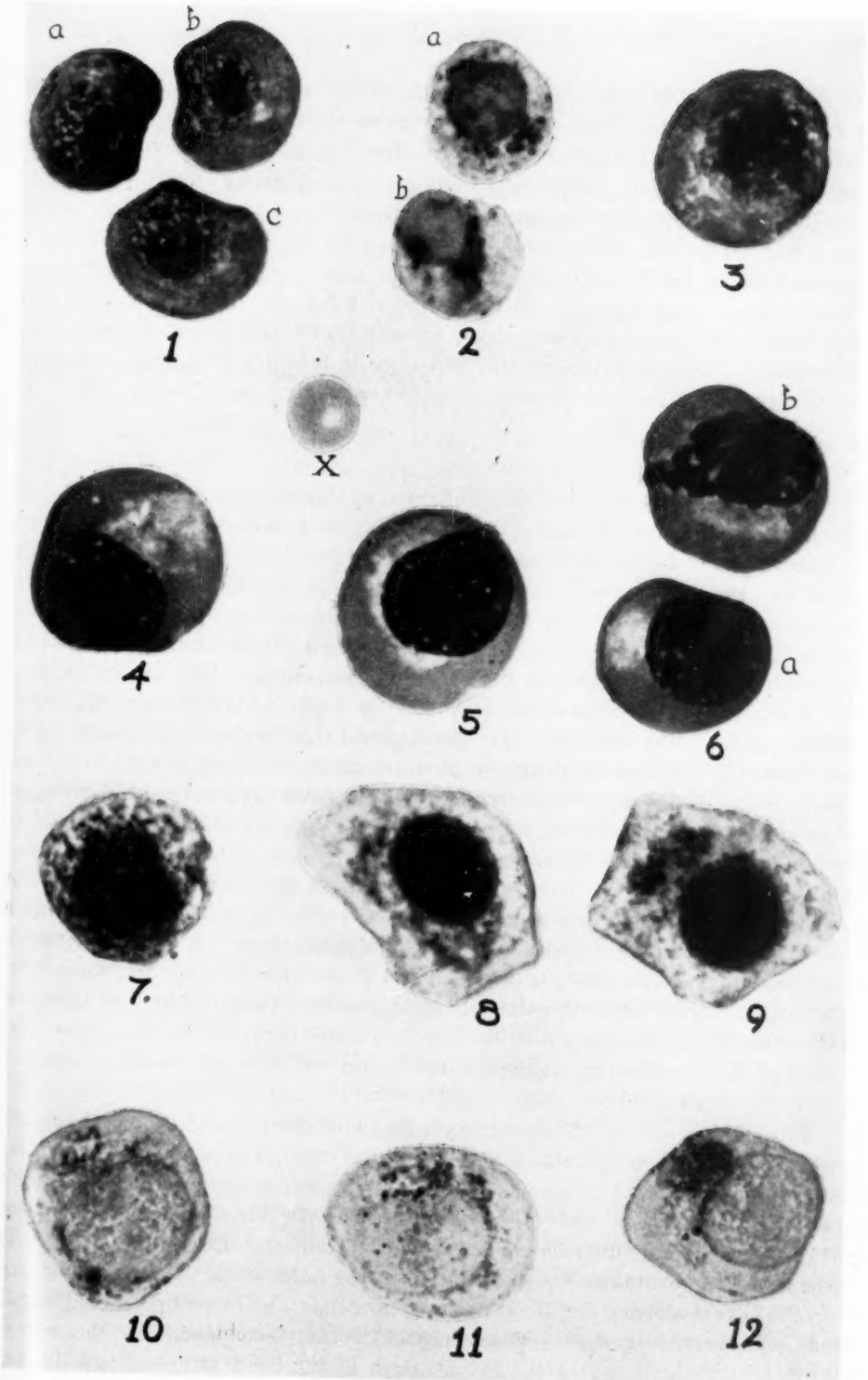

Plate I

(For legend, see opposite page.)

and *2, b,* Plate I, one wonders why so little use has been made of this technique in recent years. Studies of these preparations showed a good correlation between the distribution of mitochondria and the clear areas in the cytoplasm. In addition, the modified Schridde technique stained many more filamentous mitochondria than were ever seen with either dark-field illumination or the supravital technique. This very likely is due to the fact that the action of acid fuchsin is dependent upon the lipoidal nature of mitochondria,[26] hence this technique would bring out mitochondria with low refractive indices not seen with dark-field illumination and those without proteolytic enzymes not stained supravitally. The advantage of this technique is that dry films may be stained specifically for mitochondria and compared with dry films stained with the standard hematologic dyes.

B. Embryonic Rat Blood Cells.—

1. Dry Films of Blood From Yolk Sac of Eleven-Day Rat Embryo: As indicated in the Introduction, these studies were transferred to primitive erythroblasts of the rat embryo because of the availibility of material. This was advantageous because these cells are larger and have more cytoplasm. Careful examination revealed many bizarre arrangements of the so-called hyaloplasm. Cell *3* is of interest because of curved areas which, in one instance, appeared horseshoe-shaped. Areas which have a similar appearance have been described and illustrated by Sundberg and Downey[27] in reticular lymphocytes as representing cracks in the basophilic spongioplasm filled with clear hyaloplasm. This quite naturally implies an absence of definite structure, if we adhere to the accepted usage of the term hyaloplasm.[28] In all probability some of these light areas in other cells have been called trophospongium or canaliculi.[29] In a fair percentage of the cells examined, these areas in the cytoplasm had a very definite yellowish tinge. This perhaps would have been ignored, if it had not been recalled that Bensley[30] described extracted liver mitochondria as having a yellowish appearance. In spite of subsequent findings which indicated that a carotenoid pigment is present, it is still not known whether or not this coloration can be attributed to the stain or to the natural state of these cytoplasmic areas. Right or wrong, it fortified the evidence favoring the mitochondrial nature of the so-called hyaloplasmic areas and led to other investigations the results of which justified the original assumption.

Early in the course of these investigations it was thought that perhaps one way to solve this problem was to determine the incidences of various configurations of the so-called hyaloplasmic areas as seen in dry films stained with May-Grünwald-Giemsa and to correlate them with the incidences of known mitochondrial configurations. Primitive erythroblasts may be divided into three groups according to the distribution of the light areas. In the first group the cells have a more or less diffuse arrangement as illustrated by cell *4*, Plate I. Such cells constitute about 60 per cent of all erythroblasts. In the second group the cells have a greater concentration of the light areas around the nucleus, forming a perinuclear Hof. In general, cytoplasm between this perinuclear clear area and the cell margin is more homogeneous than that in the first type. Compare cells *4* and *5*, Plate I. The third group is made up of cells

having a fairly well-defined accumulation of clear areas more or less to one side of the nucleus, a juxtanuclear clump as shown in cell *6*. In some instances this juxtanuclear light area would be separated from the nucleus by a band of basophilic cytoplasm and in others it would be in contact with the nuclear membrane. Careful examination of these juxtanuclear light areas, as well as perinuclear areas, revealed that they are composed of many closely packed yellowish or pale cocci-shaped and short plump rodlike areas. Cells of groups two and three constitute about 30 and 10 per cent, respectively, of all primitive erythroblasts. Additional illustrations of these cells may be found in articles dealing primarily with nuclear structure[1, 31] and in a recent one concerning cell potentialities.[32]

2. Supravital Preparations of Blood From Yolk Sac of Eleven-Day Rat Embryo: In general, the observations made on the chick cells stained with janus green or pinacyanol apply equally well for rat embryonic blood so that they need not be repeated here. However, it is important to describe the staining reaction of embryonic rat cells to neutral red. Granules or vacuoles (6 to 18) of variable size usually appeared in that region of the cell referred to as the centrosphere which in most instances also represented the area with the greatest concentration of mitochondria. All granules or vacuoles were not limited to this area because many were found around the nucleus, as described by Sugiyama[5] for the chick. At the present time it is impossible to state whether or not all of the structures stainable with neutral red are identical in nature. However, there are some of these which enlarge greatly with time, while the others remain more or less the same size. Unfortunately Baker's[15] method of using neutral red, which prevents an increase in their size, was not tried. Occasionally it was impossible to decide whether or not a few mitochondria were also stained with neutral red, especially in the case of the cocci-shaped mitochondria. Practically all of the neutral red vacuoles were colored uniformly, but in rare instances they had a chromophilic periphery and a chromophobic center. It has not been determined whether or not these would eventually develop into the first type. Cell *4* has a single clear round area in the upper left portion of the cell. This is not unlike some of the neutral red vacuoles with respect to size and position. Even though the cells in the supravital preparations were not flattened to the extent that they were in dry films, the distribution of the mitochondria corresponded to the distribution of the light areas previously described. In addition to this, there was ample evidence to indicate that some of the light areas, or so-called hyaloplasm, were due to structures which stained with neutral red in the living. In view of the recent articles by Baker[15] and Worley,[33] the latter represent part of the Golgi element.

3. Permanent Mitochondrial Preparations of Blood From Eleven-Day Rat Embryo: All of the evidence thus far has indicated that the so-called hyaloplasmic areas, for the most part but not exclusively, represented negative images of mitochondria. The neutral red portion of the Golgi element contributed a very small part. If the centrioles contribute anything to the formation of these areas, it must indeed be very small, since they have not been demonstrated with certainty in any of the previous or subsequent studies. Quite

early in these investigations it was deemed necessary to correlate the distribution of mitochondria in dry films with that of the light areas demonstrable by routine hematologic stains. This quantitative approach to the problem was later abandoned in favor of a more direct one, namely, phase microscopy, which will be discussed in section *B, 6*.

Qualitative studies revealed that, in general, mitochondria of primitive erythroblasts had three types of distribution similar to the arrangement of the light areas described in sections *A, 1* and *B, 1*. Compare cells *4, 5*, and *6* with *7, 8, 9* of Plate I. For no reason other than that of convenience, Regaud's fixative was used more frequently than acetic osmic bichromate. In cells fixed with the former it was a fairly constant occurrence for the mitochondria to be thicker. This is in agreement with Baker's[15] statement that potassium bichromate causes them to round up and swell. On the other hand, the acetic osmic bichromate seemed superior for the fixation of filamentous mitochondria. Cells *8* and *9* are of particular interest because in addition to the perinuclear and juxtanuclear distribution of mitochondria, quite a few are scattered throughout the cytoplasm. As a rule more mitochondria were stained with this technique than with the supravital technique. Although much criticism has been leveled against the use of dry films for cytologic studies, especially of the nucleus, it was interesting to note that mitochondria behaved as discrete structures resisting mechanical force. The evidence for this was that they were seen as fuchsinophilic bodies in the neighborhood of damaged or smashed cells. Lazarow[34] has reported that free mitochondria from the guinea pig liver may at times retain their exact intracellular shape.

The correlation which has been described is nothing new in hematology. Forty years ago Schridde[35] maintained that the perinuclear clear Hof of lymphoblasts, as seen in sections stained with azure II-eosin or methyl green-pyronin, was nothing more than the negative image of accumulations of Altmann-Schridde granules (mitochondria in present-day terminology). In a very critical review of this subject, Klein[36] discredited Schridde's view by stating that the mitochondria were not located in the perinuclear clear zone but more peripherad. He further maintained that the perinuclear zone was an artifact. This and other polemics of that period centering around the unitarian and dualistic theories for hematopoiesis apparently caused hematologists to forget Schridde's original observation. Although the present study does not involve lymphoblasts, it seems as though both Schridde and Klein were correct to a certain extent, if a distribution of mitochondria like that in primitive erythroblasts obtains in lymphoid cells.

4. Formol Sudan Black Preparations of Blood From Yolk Sac of Eleven-Day Rat Embryo: If all of the previous observations were correct, then it should be possible to apply certain histochemical techniques in order further to analyze the true nature of the so-called hyaloplasmic areas. It has been known for some time that mitochondria contain lipoid (in general sense). Bensley[39] reported that mitochondria from guinea pig liver contain about 35 per cent total lipoid and Goerner[37] found that they ranged from 27.0 to 32.4 per cent in the rabbit liver. It has been rather paradoxical to know that in spite of this

high lipoid content, mitochondria did not stain with sudan III or IV.[30, 38] This has been attributed to the lipoid being inaccessible to sudan in a lipoprotein complex, and Bensley[30] has suggested that these negative results were due to the fact that the fat of the mitochondria was dispersed in ultramicroscopic form. In the past, one way to unmask these lipoids was to use the rather tedious procedure of Ciaccio outlined by Bowen,[29] but sudan black readily accomplishes this physically rather than chemically. Lison[14] reported the superiority of sudan black over all other similar dyes for demonstrating total fat histochemically. This is due to the fact that sudan black has a great affinity for all lipoids and readily dissolves in them. Perhaps equally important is the fact that blue blacks and black are more discernible than the various oranges, reds, etc. Sudan black also has another important advantage which has been pointed out by Baker,[15] namely, that it does not have a tendency to form connecting bridges between the structure in which it is deposited and another. It was my good fortune to have Mr. E. R. Hayes, who has been studying the histochemistry of the adrenal cortex, introduce the use of sudan black in our laboratory.

After having studied mitochondria by means of the supravital and Bensley-Cowdry techniques and dark-field illumination, it was not difficult to identify them in formol-sudan black preparations. For the most part they appeared as gray or grayish black cocci and short plump rods. Filamentous forms varied in their color from gray to a color which was almost indistinguishable from that of the surrounding cytoplasm. Since sudan black was available to all parts of the cell, it is justifiable to conclude that mitochondria within a given cell differ in their lipoid composition. In general, the distribution of the mitochondria could be divided into three main categories, namely, diffuse, perinuclear, and juxtanuclear, as illustrated in cells *10*, *11*, and *12* of Plate I. In addition to the mitochondria, there were a few spherical sudanophilic structures which constantly appeared black or bluish black. Two such structures are illustrated to the left of the nucleus in cell *12*. In most instances they appeared as sudanophilic throughout, but occasionally some with sudanophobic cores were encountered. They ranged from zero to eight per cell and were perhaps more constantly associated with the accumulation of mitochondria than any other region. In view of Baker's[15] work, these structures are in all probability related to the Golgi element and will require further study. The present evidence suggests that a clear spherical area like that in cell *4*, Plate I, represents the negative image of a portion of the Golgi element.

The sudan black preparations demonstrated that considerable sudanophilic material may be located above and below the nucleus as illustrated in cell *11*. It is quite possible that this may explain the appearance of vacuole-like structures and rarefactions in some nuclei of leukemic cells.

From a cytochemical point of view, cells *11* and *12*, Plate I, are interesting since the disposition of sudan black indicated that the perinuclear and juxtanuclear areas contain much more lipoid than the surrounding cytoplasm. An attempt was made to localize protein in this area by the use of Millon's reagent.[13] Thus far it has been impossible to demonstrate that protein is more concentrated in one part of the cell than it is in another. These observations

are contrary to those of Schleicher,[39] whose conclusions drawn from stained preparations alone have been too far-reaching. He has assumed that the Hof of pronormoblasts contains more protein than the perinuclear zone either because the fluid state of the Hof permitted protein to migrate there in the process of drying or because there is normally a greater concentration in that particular region. Although the present article deals primarily with a study of embryonic rat blood cells, sufficient material has been studied to indicate that in general the same conditions obtain for human bone marrow. In all probability some of the oxyphilic homogeneous patches in the cytoplasm which Schleicher described were the negative images of mitochondria.

Too much emphasis cannot be placed on the necessity of having good dry films for cytologic and diagnostic purposes. They should be rapidly dried and stained almost immediately for the best results. Most of us have obtained poor results after staining films which have been left overnight or longer. Quite recently some direct evidence was obtained which gave a clue to the possible nature of this process, which may rightfully be considered as degenerative. In order to study the stability of cytoplasmic structure in dry films, preparations were permitted to remain unfixed for four, six, and eight days, either in room air or in a desiccator. They were subsequently divided into three groups and stained by one of the following methods: May-Grünwald-Giemsa, Bensley-Cowdry, or formol-sudan black. There was certainly a loss of cytoplasmic detail as reported by Schleicher.[39] There was also a tendency for mitochondria to become less distinct. Fuchsinophilic and sudanophilic material appeared in portions of the cytoplasm not usually possessing these properties. The interpretation of sudan black preparations was that additional lipoid material had been made available for the dye. Perhaps this was by the breaking down of lipoprotein complexes which Lison[40] discusses under the heading of lipophanerosis. There may also be some spreading of lipoid material from the mitochondria to adjacent cytoplasmic areas. As to whether or not these processes are solely responsible for the alterations in the tinctorial qualities of cells stained with Romanowsky dyes, or a parallel phenomenon, cannot be stated at this time. In general, these changes were not as great in cells which were kept in the desiccator as they were in cells which have remained in room air.

Hematologists are beginning to find sudan black useful for studies other than those previously reported. Baillif and Kimbrough[41] studied leukocyte granules in various stages of development in both normal and pathologic blood. Eosinophilic and neutrophilic granules exhibited a sudanophilia even when they were very immature. Basophil granules were entirely sudanophobic. In our laboratory, Kibler[42] has found that Auer rods in acute leukemia are definitely sudanophilic, a property which distinguishes them from other forms of so-called azurophilic granulation including Kurloff bodies.

5. Reaction of Blood From Yolk Sac of Eleven-Day Rat Embryos to Antimony Trichloride: It already has been mentioned that quite early in these investigations the yellowish tint of the so-called hyaloplasm suggested a possible relationship of these areas to mitochondria. After this was established to a fair degree of certainly the most obvious thing to do was to deter-

mine whether or not primitive erythroblasts had carotenoid pigment localized within their mitochondria. Joyet-Lavergne[43] and Bourne[38] have applied the Carr-Price[17] reagent of antimony trichloride in chloroform to a wide variety of plant and animal tissues. Due to the fact that vitamin A produces an intense blue reaction with this reagent and carotene produces a greenish blue color, these authors were unable to determine exactly which of these carotenoids was present or whether both were. Goerner,[37] however, using chemical methods which permitted a more exact analysis of isolated mitochondria, was able to show that they contain vitamin A. In the course of studying liver, cardiac muscle, and genital glands of birds, reptiles, amphibians, and fish, Joyet-Lavergne[44] observed that some red cells showed traces of vitamin A. Since its absence was not the general rule, he conducted further studies on the blood of the skate. Red blood cells of the skate were grouped into three categories depending upon their reaction to the Carr-Price reagent. Cells belonging to the first group, which were the most numerous, had fine blue filaments near the nucleus with rare granules and short rods in the rest of the cytoplasm. Those of group two were richer in fine blue filaments and granules than group one. The last group contained cells which failed to give a blue reaction. In order to localize this reaction, parallel studies of blood with mitochondrial techniques were conducted and showed a distribution of these elements similar to the three categories previously listed. According to Joyet-Lavergne, this confirmed the general proposition that vitamin A is an essential constituent of mitochondria. By the use of fluorescence microscopy, Popper[45] concluded that the appearance of some of the fine bars or droplets in epithelial cells simulated the appearance of mitochondria.

The results of the present study on primitive erythroblasts of the rat are of interest not only because they showed that these cells give a positive reaction to the Carr-Price reagent but because it was possible to localize this reaction within a single mitochondrion. For routine determinations of the presence of carotenoid pigment the best results were obtained by examining the preparations with a 4 mm. objective. As a rule the greenish blue color reaction appeared in ten seconds and lasted for twenty to seventy seconds before it commenced to spread throughout the cytoplasm and fade. The distribution of this color reaction corresponded in general to the three types of mitochondrial arrangement. By using a half aperture on the substage condenser diaphragm or oblique lighting it was possible to localize this reaction within a single mitochondrion with the oil immersion lens. Even long filaments reacted uniformally throughout. Careful examination of a mass of mitochondria similar to those shown in cells *6*, *9*, and *12* would often reveal many of the individual elements. Such an exact localization was made possible only by all of the previous studies leading up to this point. Although Andersen and Levine[46] have described a method for differentiating vitamin A from carotene, no attempt was made to do so in the present studies. Hence, the reaction obtained in the embryonic rat cells may indicate the presence of either one or both of these substances. However, the results of Goerner's[37] work suggest that we were very likely dealing with vitamin A.

One of the most surprising findings in these experiments was that cover slip preparations of embryonic blood kept in the dark gave a positive reaction to antimony trichloride as long as four months after they were made. There did not seem to be an appreciable loss in the intensity of the color reaction. Bodansky[47] has pointed out that vitamin A in nature is quite resistant to oxidation and attributed its stability to the presence of antioxidants. Popper[45] observed that tissue sections kept in plasma or serum did not lose their vitamin A as rapidly as those kept in water. This he believed was due to the presence of some protective principle. Such a thing may very well explain why dry films of embryonic blood retained their vitamin A after four months, since the cells were surrounded by a thin film of dried plasma. For a discussion of the significance of the presence of vitamin A in mitochondria see Bourne.[26] So far it has been impossible to determine the presence of vitamin C in primitive erythroblasts.

6. Correlation of Mitochondria With the Negative Images in the Cytoplasm by Means of Phase Microscopy: Up to this point, both in the case of results reported by Schridde[35] and those previously mentioned, it has been possible to conclude that the light areas in the cytoplasm of dry films stained with Romanowsky dyes represent, for the most part, negative images of mitochondria, only by making parallel studies with several techniques and correlating the findings. Although all of the accumulated evidence has pointed in this direction, technical difficulties have made it impossible, until quite recently, to say with certainty that small groups of mitochondria or even a single mitochondrion as seen in an unstained preparation will appear as a negative image when stained with routine hematologic stains. Phase microscopy[18] has provided the answer to this problem.

Unstained dry films were usually mounted on a drop of Baker's formol-calcium fixative. The rationale for this was twofold. First, the mounting medium should be of such a nature that it would not only fix the cells to the cover slip but that it would also prevent the passage of lipoids into colloidal solution. Second, it should permit subsequent staining with either a routine hematologic stain or sudan black. The prime objective was to locate, by means of optical differentiation, filamentous mitochondria not necessarily associated with either perinuclear or juxtanuclear accumulations of these structures, and then determine whether or not they appeared in the stained preparations. By using this approach it was possible to decide definitely that the streaks, crevices, curved areas, and structures which suggest canaliculi in the basophilic cytoplasm are truly the negative images of mitochondria. For example, the horseshoe-shaped clear area of cell *3* represents the negative image of an underlying mitochondrion. Such a configuration is not an uncommon finding in primitive erythroblasts. However, it is important to realize that not all mitochondria will appear as negative in a dry fixed smear stained with one of the Romanowsky dyes. The extent to which they may be visualized is dependent upon how deeply they are located within the cytoplasm. As a rule the more superficial mitochondria located by bright contrast phase microscopy are the ones which will readily appear as negative images. The deeper ones which are even diffi-

cult to visualize with bright contrast are usually obscured by the overlying basophilic cytoplasm in routine preparations. If the mitochondria are grouped, as in perinuclear and juxtanuclear accumulations, then their representation as a negative image is practically assured in preparations subsequently stained with Wright's stain.

When unstained dry films are studied with bright-field microscopy considerable structure may be demonstrated in the cytoplasm, if the condenser diaphragm is closed about one half. As a matter of fact, cell *13,* Plate II, contains more structure than Isaacs[48] allows for stained cells which he calls megaloblasts. When these cells were studied with bright- and dark-contrast phase microscopy, details previously lost in diffraction patterns were made much more distinct. Cells *14* and *15,* Plate II, show short rodlike mitochondria and ringlike structures. The latter will be described more fully later. Cells *16* to *18,* Plate II, are of particular interest since most of the mitochondria were distributed in the same optical plane. Attention is directed to the morphology and relative position of the mitochondria in unstained cells *16* and *17.* After the same cells were dried and stained with Wright's stain, most of the filamentous forms appeared as negative images in the deeply basophilic cytoplasm (cell *18, a,* Plate II). In addition to these, the perinuclear accumulation of mitochondria and Golgi elements shown in cells *16* and *17* also appeared as negative image areas. These are undoubtedly identical with the light areas in the cytoplasm of proerythroblasts which Naegeli[25] described and illustrated without knowing their true nature. A comparison of cells *16* and *17* with cells *18, a* and *18, b,* Plate II, should be very instructive to hematologists because of their size differences. Measurements of the mean cell and nuclear diameters shows that, after fixation and staining with Wright's stain, the nucleocytoplasmic ratios have been increased slightly. In the case of *18, a* it changed from 60 to 67.4 per cent. This was due to the fact that alcoholic fixation caused a relatively greater shrinkage of the cytoplasm than that of the nucleus. In section *B, 3* it was pointed out that mitochondria in damaged cells behaved as discrete structures resisting mechanical force. In cell *18, a* the curved filamentous light area (mitochondrion) behaved in a similar fashion. Although its relative position with respect to the nuclear membrane and edge of the cell was altered, the morphology was undisturbed by shrinkage. In a subsequent article it will be shown that cells in dry films swell when placed in an aqueous medium and shrink with alcoholic fixation even after they have been flattened and dried. Cell *18, b* is of interest because of the diffuse distribution of the light areas which correspond to the structures illustrated in cells *16* and *17,* Plate II.

One of the most striking things revealed through phase microscopy was the high degree of organization present in the cytoplasm of primitive erythroblasts after they had been smeared and dried rapidly. A false impression is obtained if cells like those illustrated in *8, 9, 11,* and *12,* Plate I, are studied with one technique alone. The impression that mitochondria are more or less heaped about the nucleus was discredited through the use of phase microscopy which revealed that they have a more general distribution and that there are some forms which do not stain readily with either acid fuchsin or sudan black. This

very likely is due to the fact that the results of those techniques depend upon the fatty nature of mitochondria[26] which is variable, and the heat treatment may have caused some filaments to break up. In spite of all the structure revealed through phase microscopy, at no time was it possible to locate anything which even approximated what we designate as a "classical Golgi net or apparatus." Furthermore, it has also been impossible to locate in dividing cells a structure which could be called a centriole.

Much valuable information was obtained by combining the formol-sudan black technique with dark contrast phase microscopy. According to Lison[49] and Baker,[15] whatever appears blue black consists of lipoids. Hence, mitochondria must vary in their lipoid content because they appear as grayish structures and some, especially the filamentous forms, must contain a minimal amount. When such forms have been suspected of being located within a certain cytoplasmic area, the use of phase microscopy has made their presence a certainty. In section *B*, *4* it was mentioned that the sudanophilic solid spheres and those with a sudanophobic core were probably related to the Golgi element. With the aid of dark-contrast phase microscopy it was possible to determine that in addition to these forms there are sudanophilic rings which pass around sudanophobic cores in a manner quite similar to that described by Baker.[15] They also resembled osmiophilic platelets of plant cells.[49] This finding tends to strengthen the view that the reason for not being able to locate a classical Golgi apparatus within primitive erythroblasts is probably not due to the fact that it was not preserved but rather that it does not exist as such. All of this will require further study.

7. The Action of Ribonuclease on Basophilic Cytoplasm: As a conclusion to all of the preceding sections which have dealt with the nature of the light areas in basophilic cytoplasm, it seems appropriate that the latter should also be analyzed in dry films. Basophilia of lymphocytes was identified by Brachet[50] as ribonucleoprotein by digesting sections of the spleen in a crude preparation of ribonuclease. Recently, Wislocki and Dempsey[51] have shown that the basophilia of definitive erythroblasts in sections of monkey's bone marrow was abolished

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PLATE II

Cells *13* to *17* were mounted in 10 per cent formalin and photographed at a magnification of 1610 diameters. Cells *18*, *a*, and *18*, *b*, were mounted in clarite and photographed at a magnification of 1625 diameters.

13, Unstained cell as seen with bright-field microscope at about half aperture. Although there is considerable cytoplasmic structure in this cell, the detail is lost in diffraction patterns.

14, Bright contrast with 0.24A + 0.2 λ diffraction plate. Note the rod and ring forms to the right of the nucleus. In sudan black preparations the rings appeared sudanophilic and are probably related to the Golgi element.

15, Dark contrast with 2.3B – 0.33 λ diffraction plate. This was focused at a slightly lower level than cell *14* in order to demonstrate structures to the left of the nucleus which appear as dark rods. Ring forms previously described may also be seen to the right of the nucleus.

16, Unstained cells in bright contrast with 0.24A + 0.2 λ diffraction plate. Note the curved and straight filamentous mitochondria in the upper half of cell *a*. Ring forms near the nucleus are also present.

17, Same cells as *16* in dark contrast with 2.3B – 0.33 λ diffraction plate. Note the shape and relative position of the mitochondria in the upper half of cell *a*.

18, Same cells as in *16* and *17* after drying and staining with Wright's stain. Bright-field microscopy. The filamentous mitochondria appear as negative images in the basophilic cytoplasm. Although there has been considerable shrinkage, the morphology of the light areas is practically identical with that of the mitochondria demonstrated by phase microscopy.

The phase photo-micrographs were furnished through the courtesy of the Research Division, American Optical Company, Scientific Instrument Division, Buffalo, N. Y., and the plates were arranged by M. D. Diedrich, Director of Medical Illustration, University of Buffalo.

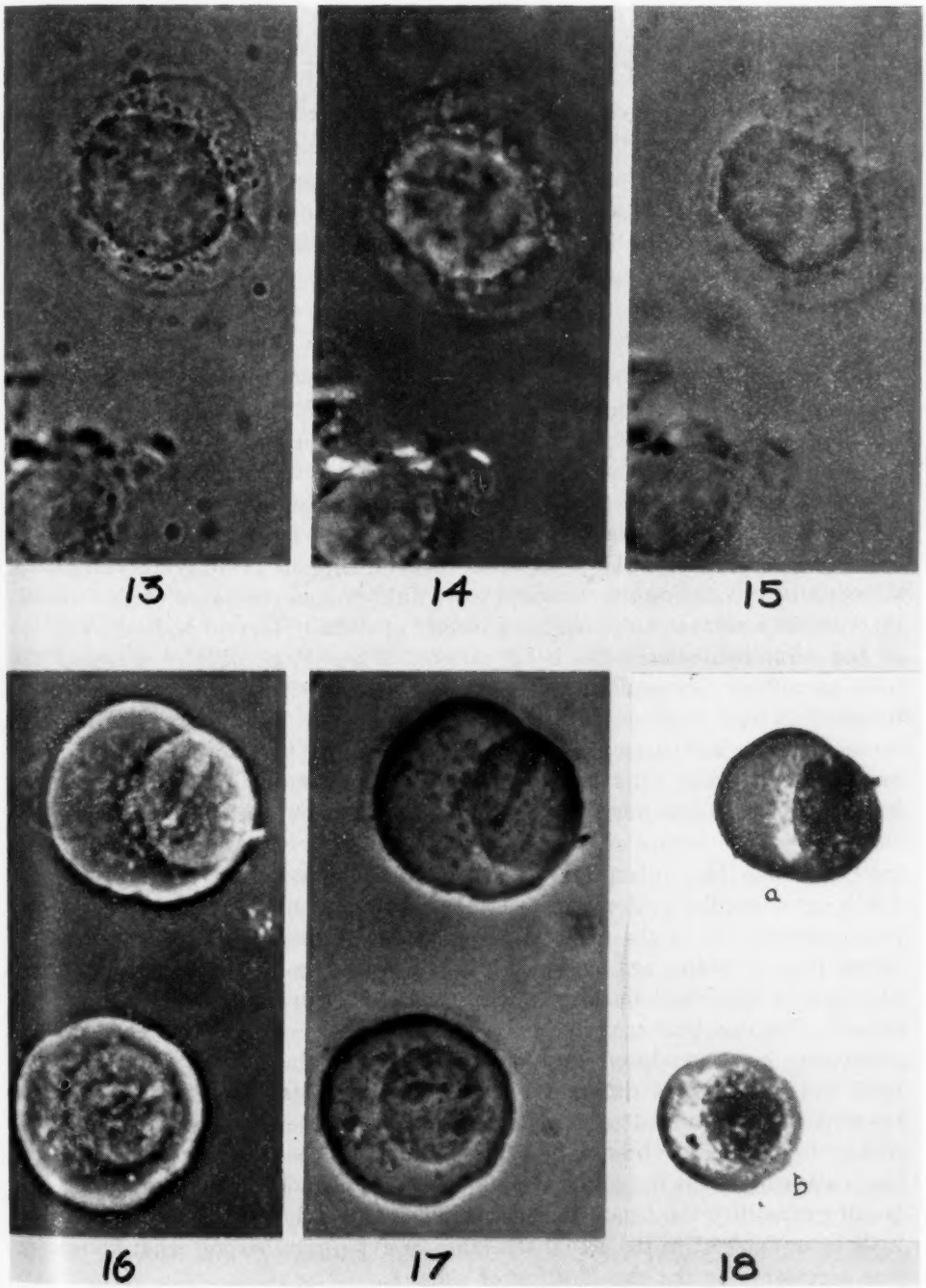

Plate II

(For legend, see opposite page.)

after digesting them in a solution of crystalline ribonuclease. In our laboratory, cover slip preparations of dry films of embryonic blood have also been successfully treated with a similar solution of crystalline enzyme* and the basophilic material in the cytoplasm and nucleoli has been identified as ribonucleoprotein. These preparations were fixed briefly in Zenker-formol and controls were incubated in buffer solution. Either methyl green-pyronin or Wright's stain were used as counterstains. Cytoplasm of digested cells stains quite acidophilically with the latter. At the present it is impossible to state how much of this reaction may be attributed to hemoglobin which has been uncovered.

DISCUSSION

It is impossible in the space allotted to discuss adequately all of the ramifications of a problem which was started over seven years ago as merely an investigation of the pale or light cytoplasmic areas of primitive erythroblasts. It has grown and extended well beyond the limits of pure morphology due to the development and application of newer histochemical and optical techniques. All of the evidence presented indicates that it is no longer logical to ascribe a hyaloplasmic nature to the clear areas of cytoplasm in primitive erythroblasts since, contrary to being the clear and more fluid ground substance of protoplasm, they actually represent the negative images of definite formed bodies. The use of the term hyaloplasm has been varied. When Pappenheim[21] adopted the term paraplasm (hyaloplasm) he did so not only because accurate descriptive terminology was lacking in the field of morphologic hematology, but also because most cytologists were still using this term in the sense originally employed by Leydig.[52] To say the least, the two terms, spongioplasm and hyaloplasm, have served a useful purpose in the absence of more specific information regarding the true nature of these substances.[1, 6, 25, 27] However, most cytologists have forsaken this interpretation of cytoplasmic structure in favor of one which considers the hyaloplasm to be the continuous phase of a polyphasic colloidal system. It is the clear, apparently homogeneous ground substance in which formed bodies are suspended.[26, 28, 53, 54] When Pappenheim[55] published his work he continued to use the term paraplasm (hyaloplasm) in its original sense.[55] During this same period Schäfer[52] deplored the fact that certain cytologists had introduced confusion into the terminology by employing the term hyaloplasm in a different sense. In spite of the fact that most hematologists have continued to use the terms spongioplasm and hyaloplasm (paraplasm) to describe the basophilic cytoreticulum and the lighter interspongioplasmic material present in lymphoid cells, biologists abandoned this usage long ago. In all probability the hematologist continued to use these terms because he had little or no interest in the actual structure of cytoplasm beyond what knowledge was necessary for the classification of cells.

Perhaps one of the most important findings in the present investigation is that since 1880 hematologists have been making dry film preparations which

*Crystalline ribonuclease was kindly furnished by Dr. E. A. Sharp, Department of Clinical Investigation, Parke, Davis & Company, Detroit, Mich.

preserve cytoplasmic detail with an extraordinary degree of accuracy. It seems to me that hematologists should not be castigated so much for using the dry film method of studying blood,[56] as for not having endeavored to find out the nature of some of the things which they have repeatedly described and perhaps argued about. To be sure, new techniques should be devised and used but not to the extent that the older ones are not developed and utilized to their optimum. Cunningham and Tompkins[23] pointed out that the methods of Ehrlich and Romanowsky are not capable of demonstrating certain specific data which the supravital technique reveals. This is only partially true in the case of primitive erythroblasts from the chick and rat, for we now know that a fairly accurate estimate of the mitochondrial distribution may be obtained from a study of good dry smears alone. This reevaluation of what can be seen in dry films and its interpretation increases the usefulness of this method for studying the influence of antianemic substances transmitted across the placenta.[7, 8] Cell size, nuclear structure, and the general mitochondrial distribution may all be studied in a single properly prepared dry film of embryonic blood.

The use of dry films of the embryonic yolk sac was introduced by Kirschbaum[11, 31] primarily for the purpose of studying the nuclear structure of mammalian embryonic blood cells and comparing these with the megaloblasts of pernicious anemia. Later, this material was found suitable for studying the influence of antipernicious anemia principle on embryonic erythropoiesis.[7, 8, 57, 58] The present article has revealed that this material is particularly suitable for investigating the cytochemistry of a single cell. Although the latter has been limited to only a few techniques, it is hoped that additional ones will be applicable to this material and that our knowledge will be extended in this new and interesting field. The method of approach outlined in the preceding pages may very well be applied to other cell types with slight modifications necessary to suit the individual problem.

At the present time it is impossible to predict what practical applications in clinical hematology will arise from knowledge that negative images of mitochondria are demonstrable with routine hematologic stains. Perhaps it may lead to a differentiation between macronormoblasts found in the marrows of patients with hepatic cirrhosis and those in the marrows from patients with various hemolytic anemias. It will be interesting to determine whether or not vitamin A is present in the former. Finally, megaloblasts must now be restudied in light of these recent findings.

SUMMARY

1. Blood from the yolk sacs of eleven-day rat embryos was studied by means of various cytologic, cytochemical, and optical techniques.

2. Freshly dried films of primitive erythroblasts preserve cytoplasmic detail to an extraordinary degree.

3. Light areas in basophilic cytoplasm previously described as hyaloplasm or paraplasm represent, for the most part, the negative images of underlying mitochondria.

4. Cytochemical techniques reveal that the light areas (mitochondria) are relatively rich in lipoid material and contain a carotenoid pigment, probably vitamin A. Cytoplasmic basophilia is due to ribonucleoprotein.

5. Phase microscopy makes it possible to study structures in unstained blood cells which are usually hidden in routine preparations.

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INDUCTION OF LEUKEMIA IN EIGHT INBRED STOCKS OF MICE VARYING IN SUSCEPTIBILITY TO THE SPONTANEOUS DISEASE

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THE incidence of spontaneous leukemia in inbred mice is dependent on the genetic constitution of the strain. If a chemical or physical agent is to be considered "leukemogenic" then its administration must result in (1) an increase in the incidence of leukemia among test as compared with control mice of the same stock, (2) an accelerated onset of the disease, or (3) both effects.

Using these criteria, agents of three general types, two chemical and one physical, have been considered to be leukemogenic. The chemical agents are first carcinogenic hydrocarbons, such as carcinogenic tar,[8] methylcholanthrene,[11, 24, 27, 32] benzpyrene,[24, 31] and 9, 10-dimethyl-1, 2-benzanthracene,[17, 26] and second various estrogens.[14, 15] Ionizing radiations such as x, gamma, and beta rays represent the physical agents capable of inducing this effect.[7, 16, 17]

Included under the term "leukemia" in mice are systemic lymphatic and myelogenous leukemia and both localized and metastatic lymphosarcoma. Certain high leukemia stocks develop all these disease types spontaneously.[23] The induced disease has usually been lymphoid in our experience, except in those instances where the stock of mice develops myeloid leukemia spontaneously.[25] Morphologically and clinically these human and mouse diseases are very similar.[9] Response to therapeutic agents is comparable in both species.[5, 6, 12, 13]

Susceptibility to the induction of leukemia cannot always be correlated with susceptibility to spontaneous leukemia.[8, 21, 33] Furthermore, x-rays may induce leukemia in stocks which are refractory to the chemical carcinogens.[21] This report represents an analysis of these phenomena in eight inbred stocks of mice.

MATERIALS AND METHODS

Control groups for each stock of mice (except C57 Black) were observed by us. The strains used for experiments were dba-subline 212, Bagg albino, CBA, C3H, Strong A, NH, C57 Black, and F. In each stock (except C57 Black) the offspring of the controls were used as test animals. All mice were fed a diet of Purina fox chow and were permitted to breed to the extent that treatment with the leukemogenic agents allowed. A total of 1,400 mice were observed; the number used for each experiment is indicated on the bar graphs (Figs. 1 to 5) and tables.

Mice were placed on experiment at 10 weeks of age. Methylcholanthrene was administered by skin-painting, the carcinogen being dissolved in benzene as a 0.25 or 0.5 per cent solution. The solution was applied with a camel's hair brush (⅜ by ⅝ inches), a different

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This investigation has been aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research, the National Cancer Institute, and the Cancer Fund of the Graduate School of the University of Minnesota.

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area of the skin receiving the carcinogen with each painting. Animals were painted three times weekly with the weaker and twice weekly with the stronger solution. Estrogen was administered to Bagg albino and CBA mice in the form of a subcutaneous pellet of estrone* and to C3H animals by a single subcutaneous injection of 50 gamma weekly (throughout the experiment) of estradiol dipropionate in 0.1 c.c. of sesame oil. X-rays were administered by whole body irradiation, 140 kv., 30 cm. distance, 2 mm. aluminum filter. The mice were kept in a cardboard container while receiving radiation. Roentgens in air and interval of application are indicated on the bar graphs and their legends (Figs. 1 to 5) and in the tables (Tables I to VIII).

Leukemia was diagnosed by both gross and microscopic examination. The disease was detected in most of the animals ante mortem by physical examination.

RESULTS

Strain dba-Subline 212 (Table I and Fig. 1).—Following only eighteen paintings of a 0.25 per cent solution of methylcholanthrene in benzene, there was an increase in the total incidence of leukemia and an acceleration in the onset of the disease (Fig. 1, *b*). If the carcinogen was applied three times weekly and this treatment was continued until the animal was sacrificed because death seemed imminent, these effects were accentuated (Fig. 1, *c*). X-rays were also leukemogenic for this strain, but not to the same degree as methylcholanthrene (Fig. 1, *d*, *e*, and *f*). In spite of the fact that in this stock mammary cancer occurs spontaneously (Table I), and its onset was accelerated by the action of methylcholanthrene, and some females probably died as a result before leukemia developed, the incidence of leukemia was consistently higher in females (Table I). This suggests that female animals of this stock may be more sensitive than males to the development of both spontaneous and induced leukemia. Other investigators have noted that in certain stocks females develop leukemia more readily than males[3] and the incidence may be reduced by ovariectomy,[30, 34] although this phenomenon is not universal.[20]

It may be concluded that strain dba-212 is moderately susceptible to the development of spontaneous leukemia. Upon administration of methylcholanthrene, or exposure to x-rays in appropriate doses, leukemia appeared earlier

TABLE I. STRAIN DBA—SUBLINE 212

TREATMENT	NUMBER OF MICE			NUMBER WITH LEUKEMIA			NUMBER WITH MAMMARY CANCER*		NO LEUKEMIA OR MAMMARY CANCER SKIN CANCER
	TOTAL	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL	CANCER PLUS LEUKEMIA	
None	111	47	64	13	5	8	38	3	0
MC†—X18—0.25%	27	21	6	9	3	6	4	4	5
MC†—cont—0.25%	65	26	39	33	12	21	24	8	10
X-rays—200 r-X9 biweekly	29	15	14	6	1	5	0	0	0
X-rays—100 r-X11 weekly	38	28	10	13	8	5	1	0	0
X-rays—50 r-X32 weekly	24	11	13	6	2	4	2	0	0

*Females.
†Methylcholanthrene.

*Estrone pellets were supplied by Parke, Davis & Company, Detroit, Mich., through the courtesy of Dr. D. K. Kitchen.

in life than in untreated mice of this strain, and the incidence of the disease was increased. Susceptibility to the leukemogenic action of estrogenic hormone could not be determined because this stock does not tolerate the doses of estrogen known to be necessary for leukemogenesis in susceptible strains.

Strain CBA (Table II and Fig. 2).—Five out of seventy-six untreated CBA mice developed leukemia spontaneously (Fig. 2, *a*). When treated with methylcholanthrene none of thirty-four animals developed leukemia (Fig. 2, *b*).

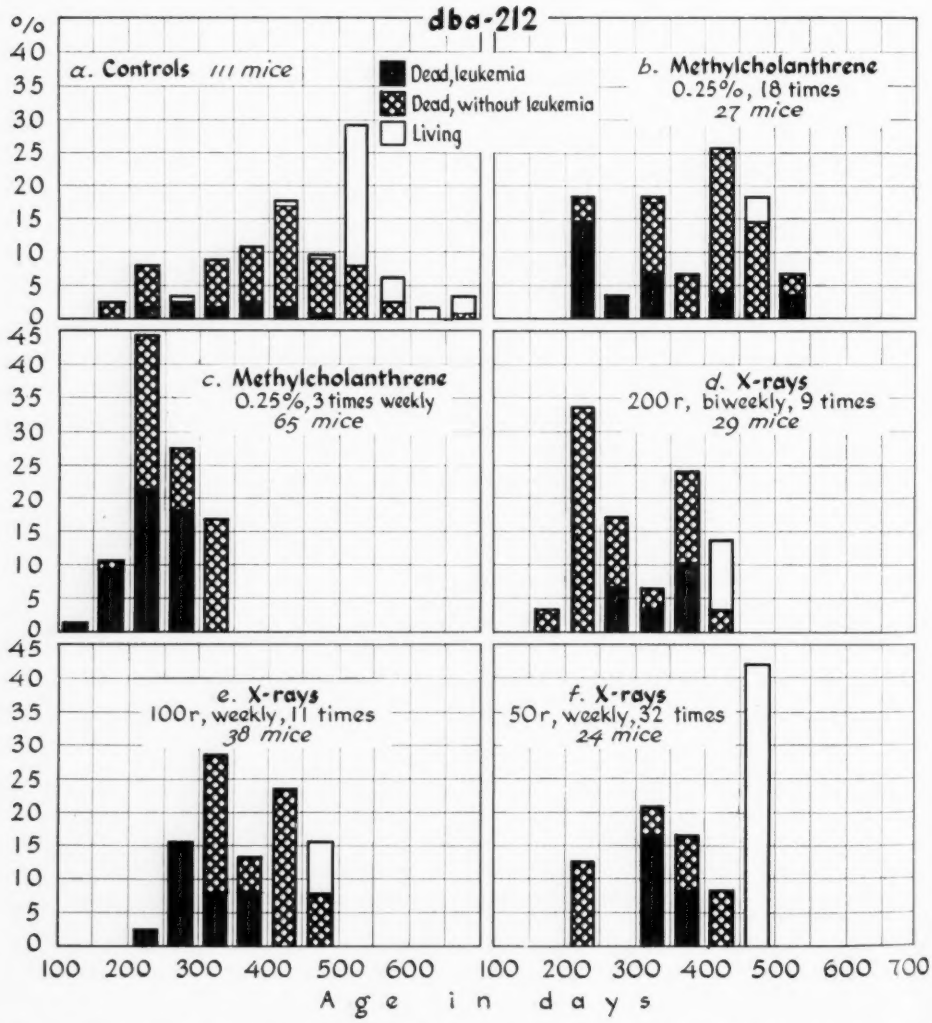
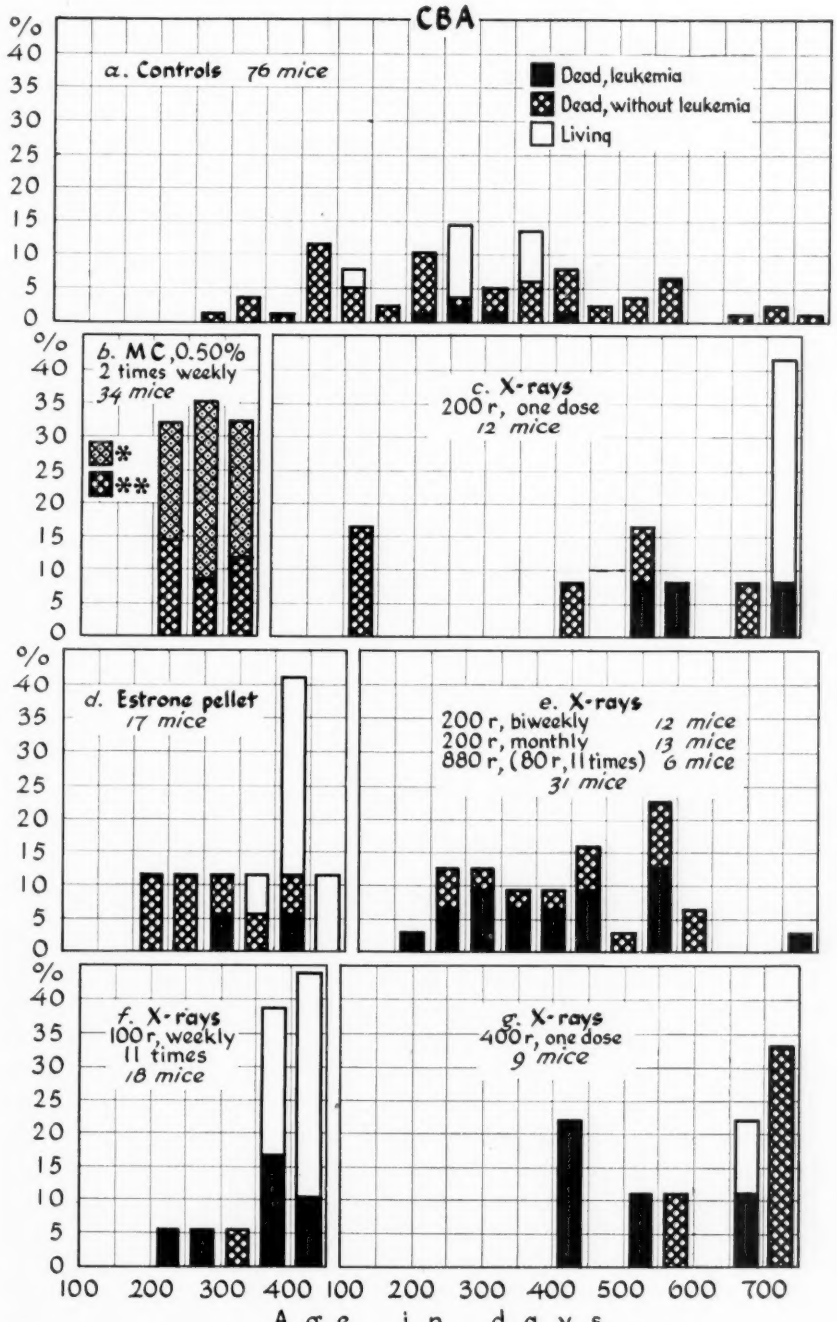


Fig. 1.—Illustrates the incidence of leukemia in controls of the dba-212 strain and in mice of this stock which were treated with methylcholanthrene or exposed to x-rays.

Because of the development of skin cancer resulting from the action of methylcholanthrene (Table II), none of these mice survived beyond 350 days of age. However, if methylcholanthrene were leukemogenic for this strain leukemia should have appeared before this age (compare Figs. 1, *c* and 2, *b*). Although



*Indicates "dead, without leukemia"; animals received MC 0.50% plus 200 r of x-rays given on the day preceding the first skin painting.

**Indicates "dead, without leukemia"; animals received only 0.50% MC.

Fig. 2.—Illustrates the incidence of leukemia in control CBA mice and in animals of the same stock which were treated with methylcholanthrene, exposed to x-rays, or treated with estrogenic hormone.

CBA mice were refractory to the leukemogenic action of methylcholanthrene, x-rays proved to be a potent leukemogen (Fig. 2, *e*). A single dose of 200 or 400 *r* is probably above the threshold range for this strain, although such doses were not as effective as much higher doses in inducing leukemia (compare Fig. 2, *c* and *g* with *e*). Estrone induced leukemia in two out of seventeen mice (compare Fig. 2, *a* and *d*). In contrast to the dba strain leukemia was not induced more readily in female than in male animals (Table II). The development of mammary cancer was not a complicating factor in this stock.

TABLE II. STRAIN CBA

TREATMENT	NUMBER OF MICE			NUMBER WITH LEUKEMIA			NUMBER WITH SKIN CANCER
	TOTAL	MALE	FEMALE	TOTAL	MALE	FEMALE	
None	76	26	50	5	1	4	0
MC*—0.5%	34	16	18	0	0	0	31
X-rays—200 r one dose	12	1	11	3	0	3	1
X-rays—200 r-X9 biweekly, once a month, or 880r†	31	19	12	18	12	6	1
X-rays—100 r-X11 weekly	18	7	11	7	4	3	0
X-rays—400 r one dose	9	6	3	4	2	2	0
Estrone pellet	17	8	9	2	1	1	0

*Methylcholanthrene.
†80 r daily on eleven successive days with no further treatment.

TABLE III. STRAIN BALB

TREATMENT	NUMBER OF MICE			NUMBER WITH LEUKEMIA			NUMBER WITH SKIN CANCER
	TOTAL	MALE	FEMALE	TOTAL	MALE	FEMALE	
None	77	27	50	1	0	1	0
MC*—0.25 and 0.5% (Fig. 3, *b*)	50	25	25	0	0	0	40
X-rays—200 r-X9 monthly	16	5	11	10	1	9	0
Estrone pellet	16	16	0	7	7	0	0

*Methylcholanthrene.

Strain Balb (Table III and Fig. 3).—Although a large number of the seventy-seven control animals are still alive, those living have reached sufficient age (Fig. 3, *a*) to serve as controls for the induction of leukemia in this stock. Only one case of leukemia has appeared among the controls. When treated with methylcholanthrene, although a large per cent of the mice developed skin cancer (Table III), these animals were of such an age (Fig. 3, *b*) that cases would have appeared in this group if methylcholanthrene were leukemogenic for this stock. When 200 r of x-rays were given monthly for nine successive months, ten out of sixteen animals developed the disease (nine of eleven females and one of five males). Estrone was almost as potent a leukemogen as x-rays from the standpoint of the total number of leukemias induced and was more active from the standpoint of latent period (compare Fig. 3, *c* and *d*). Only male mice were tested with estrone pellets, since females of this stock develop pyometra and all die within 200 days after implantation of the pellet.

Thus, Bagg albino mice were relatively refractory to the development of spontaneous leukemia, and proved to be absolutely refractory to the leukemogenic action of methylcholanthrene, but susceptible to x-rays or estrone as leukemia inciters.

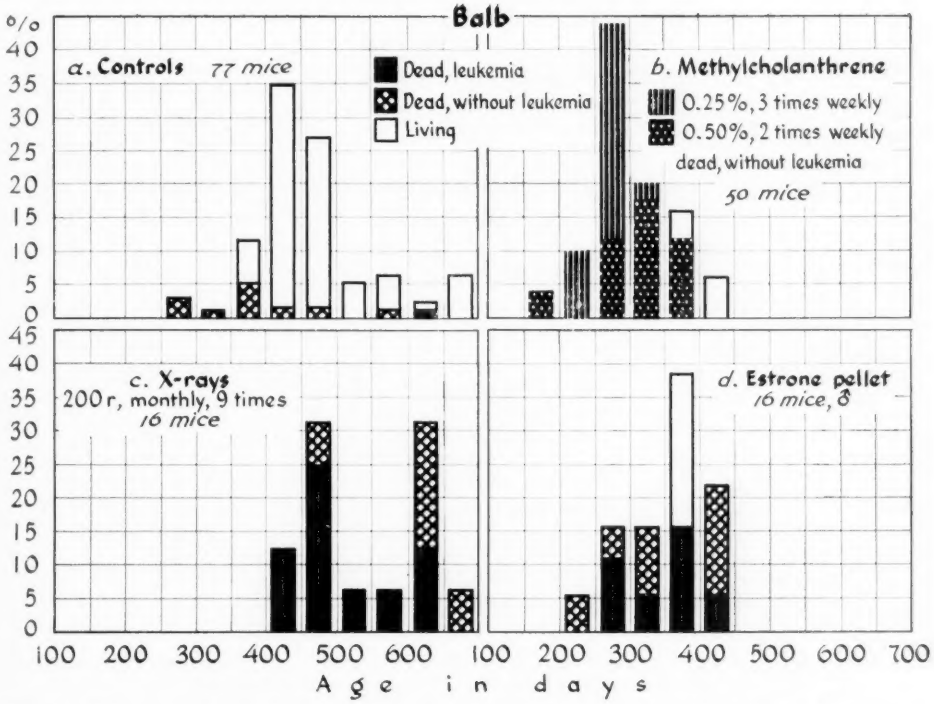


Fig. 3.—Illustrates the incidence of leukemia in untreated Bagg albino mice and in groups treated with methylcholanthrene, x-rays, or estradiol dipropionate.

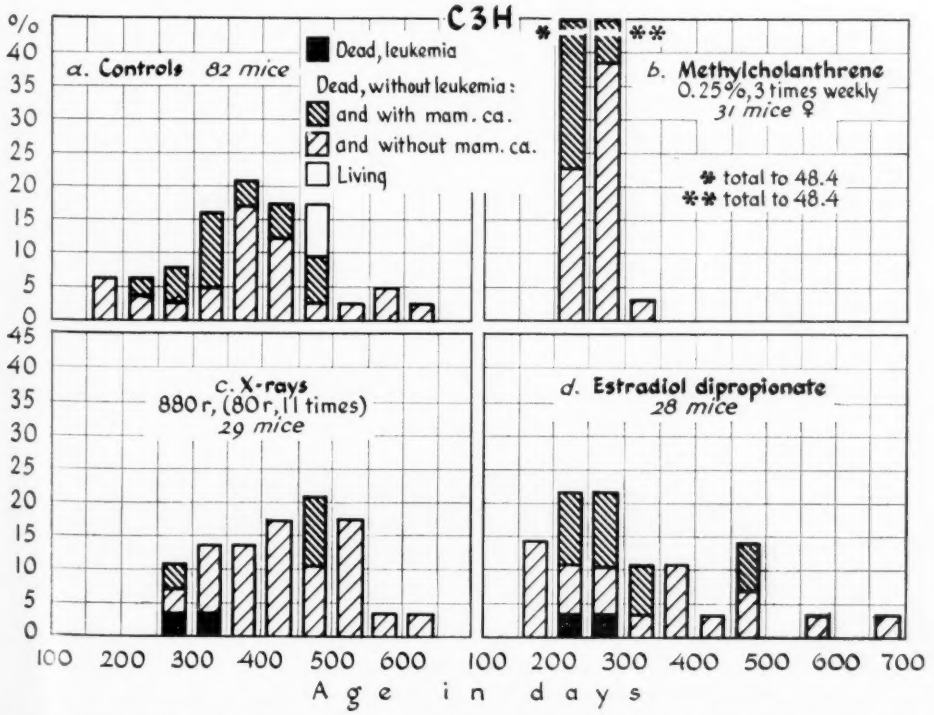


Fig. 4.—Illustrates the incidence of leukemia in control C3H mice and in mice of the same stock which were treated with methylcholanthrene, x-rays, or estradiol dipropionate.

TABLE IV. STRAIN C3H

TREATMENT	NUMBER OF MICE			NUMBER WITH LEUKEMIA			NUMBER WITH MAMMARY CANCER*		NO LEUKEMIA OR MAMMARY CANCER SKIN CANCER
	TOTAL	MALE	FEMALE	TOTAL	MALE	FEMALE	TOTAL	CANCER PLUS LEUKEMIA	
None	82	23	59	0	0	0	30†	0	0
MC‡—cont—0.25%	31	0	31	0	0	0	11§	0	18
X-rays—880 r	29	14	15	2	1	1	5	0	0
Estradiol dipropionate	28	15	13	2	1	1	11	0	0

*Females.

†Thirty out of thirty-nine mice with the mammary cancer "milk-agent" developed mammary cancer.

‡Twenty-three of the thirty-one females in this group lacked the mammary cancer "milk-agent."

§Methylcholanthrene.

*Strain C3H** (Table IV and Fig. 4).—No leukemias were observed in eighty-two control mice, nor was leukemia induced in any of thirty-one animals painted with a 0.25 per cent solution of methylcholanthrene in benzene. In a group of 29 C3H mice which received a total of 880 r of x-rays (80 r on eleven successive days), two animals developed leukemia. The same number of leukemias appeared in a group of twenty-eight animals which received 50 gamma weekly of estradiol dipropionate. In all groups a considerable number of females developed mammary cancer (Table IV). As a result of painting with methylcholanthrene skin cancer was a cause of death so that very few mice treated with the carcinogen survived beyond 300 days of age.

The C3H stock of mice is thus a low-leukemia strain which was refractory to the leukemogenic action of methylcholanthrene, but probably somewhat susceptible to the leukemia-inciting action of x-rays and estradiol dipropionate. X-rays[17] and estrogens[14] have been demonstrated by other investigators to induce leukemia in C3H mice. In a previous experiment[25] C3H animals were refractory to methylcholanthrene.

Strong A Strain (Table V and Fig. 5, *a* and *b*).—In the present experiments x-rays were the only leukemogenic agent studied. Six cases of leukemia (Fig. 5, *b*) appeared among fifty-eight mice which received radiation with x-rays, whereas there were no cases in the group of ninety-two controls. Thus, although leukemia did not appear in untreated mice of the Strong A strain, these mice were moderately susceptible to the leukemogenic action of x-rays. In previous studies methylcholanthrene did not induce leukemia in this stock[21] and estrogens were probably only very weakly leukemogenic.[14]

TABLE V. STRAIN STRONG A

TREATMENT	NUMBER OF MICE			NUMBER WITH LEUKEMIA			NUMBER WITH MAMMARY CANCER
	TOTAL	MALE	FEMALE	TOTAL	MALE	FEMALE	
None	92*	42	50	0	0	0	35
X-rays†	58‡	27	31	6	1	5	5

*Eight died of liver abscess.

†200 r-X9 either biweekly or monthly, or 400 r in one dose.

‡Fourteen without leukemia died of liver abscess.

*C3H and C57 Black mice were supplied by Dr. John J. Bittner, University of Minnesota Medical School.

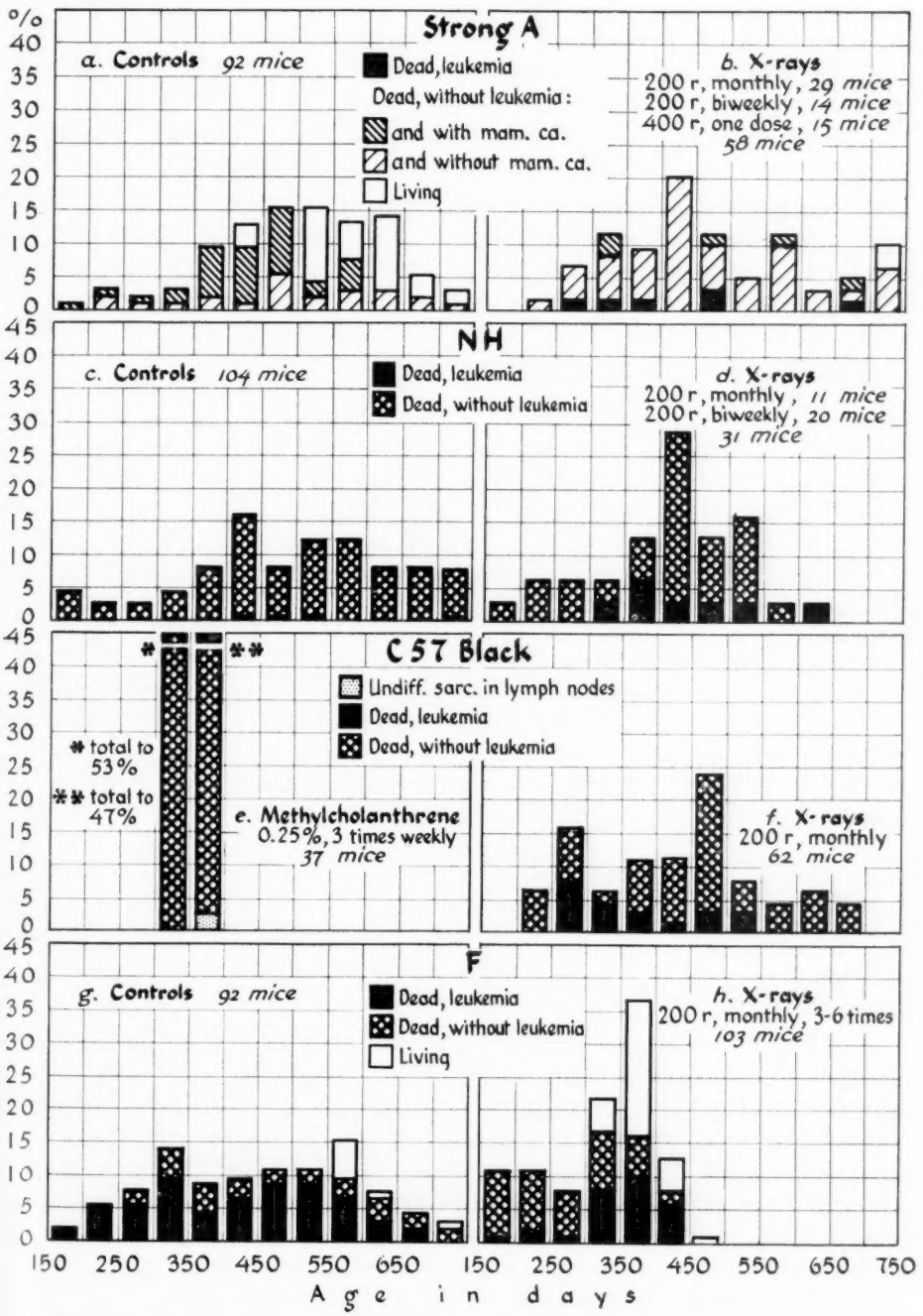


Fig. 5.—Illustrates incidence of leukemia in: *a* and *b*, Control and x-radiated Strong A mice; *c* and *d*, control and x-radiated NH mice; *e* and *f*, methylcholanthrene-treated and x-radiated C57 Black mice; *g* and *h*, control and x-radiated F mice.

Strain NH (Table VI and Fig. 5, *c* and *d*).—Four cases of leukemia appeared in the group of 104 controls and seven cases in thirty-one animals receiving x-rays. This stock did not tolerate estrogens well and methylcholanthrene was not tested.

Strain C57 Black (Table VII and Fig. 5, *e* and *f*).—These mice were refractory to induction of leukemia by methylcholanthrene, but developed leukemia following radiation with x-rays (fifteen out of sixty-two mice). Methylcholanthrene-treated mice lived to be from 300 to 400 days of age; by 400 days of age, nine of the fifteen cases of leukemia induced by x-rays had appeared. This is a low-leukemia stock which was refractory to estrogenic hormone as a leukemia incitor.[14] Susceptibility to the leukemogenic action of x-rays has been reported previously in this stock.[16]

TABLE VI. STRAIN NH

TREATMENT	NUMBER OF MICE			NUMBER WITH LEUCEMIA		
	TOTAL	MALE	FEMALE	TOTAL	MALE	FEMALE
None	104	62	42	4	2	2
X-rays—200 r-X9 monthly or biweekly	31	7	24	7	0	7

TABLE VII. STRAIN C57 BLACK

TREATMENT	NUMBER OF MICE			NUMBER WITH LEUKEMIA			NUMBER WITH SKIN CANCER
	TOTAL	MALE	FEMALE	TOTAL	MALE	FEMALE	
MC*—cont—0.25%	37	0	37	1?†	0	1?†	20
X-rays—200 r-X9 monthly	62	26	36	15	9	6	0

*Methylcholanthrene.
†Undifferentiated sarcoma of lymph nodes.

TABLE VIII. STRAIN F

TREATMENT	NUMBER OF MICE			NUMBER WITH LEUKEMIA			NUMBER LIVING
	TOTAL	MALE	FEMALE	TOTAL	MALE	FEMALE	
None	92	42	50	57	26	31	7
X-rays—200 r-X3 to X6—monthly	102	55	47	29	17	12	29

Strain F (Table VIII and Fig. 5, *g* and *h*).—This is a high leukemia stock (Fig. 5, *g*) in which leukemia appeared precociously when the mice were skin painted with methylcholanthrene.[24, 25] This strain did not tolerate x-rays well in the doses given (in Fig. 5, *h* note deaths without leukemia at an early age). The relatively poor general status of these animals following radiation might account for the fact that x-rays failed to increase the incidence of leukemia during the first year of life (Fig. 5, *g* and *h*). The doses of x-rays used did not appreciably decrease the expected incidence of leukemia for this strain. Strain F mice do not tolerate estrogens in leukemogenic doses.

DISCUSSION

Evidently multiple agents (carcinogens, x-rays, estrogens) are capable of inducing leukemia in mice. The effectiveness of each agent depends on the

genetic constitution of the stock involved (Table IX). Although the actual mechanism of induction might be the same for each agent, specific genes probably determine whether the strain will respond to the particular stimulus. It would appear that leukemia may represent one type of neoplastic response to any one of several inciting agents.

X-rays represent the only agent which might be considered to be almost universally leukemogenic for mice. However, the degree of responsiveness to this agent varies from strain to strain. Estrogens are probably second in general effectiveness, and the carcinogenic hydrocarbons third, although when leukemogenic the last-mentioned represent the most potent leukemogen. Susceptibility to spontaneous and carcinogen-induced leukemia can be correlated (Table IX). However, some strains with a very low incidence of spontaneous leukemia developed a high incidence following treatment with carcinogenic chemicals.[8, 33] Benzol has been reported to induce leukemia in mice, but the experiments were not conducted under controlled conditions using inbred stocks.[28, 29] When dba mice were skin painted with benzene alone the incidence of leukemia did not differ from that found in controls.[32]

TABLE IX. PRINCIPLE OF MULTIPLE LEUKEMOGENIC FACTORS IN MICE

STRAIN OF MICE	PRECIPITATING AGENT			
	CARCINOGENIC HYDROCARBON	X-RAYS	ESTROGENS	UNKNOWN FACTORS OF SPONTANEOUS LEUKEMIA
dba-212	++++	++	Poor tolerance	+
CBA	-	++++	+	±
Balb	-	++++	+++	-
C3H	-	±	±	-
A	-	+	±	-
NH	Not tested	++	Poor tolerance	±
C57	-	++	-	-
F	+++	Poor tolerance - ?	Poor tolerance	++++

±, 3 to 10% leukemia.
+, 10 to 15% leukemia.
++, 15 to 35 % leukemia.
+++, 35 to 50% leukemia.
++++, More than 50% leukemia.

All three proved leukemogens also induce cancer in mice. The carcinogenic hydrocarbons, of course, induce neoplasms of many types (skin cancer, sarcomas, lung tumors). X-rays induced ovarian tumors readily[10] and among irradiated mice there was an increase in the incidence of miscellaneous tumors.[10, 32] The estrogens have been demonstrated to induce cancer of the uterine cervix[1] and testicular tumors of mice.[18] None of the other steroid hormones has been proved to be carcinogenic.

The fact that carcinogens are also leukemogens lends support to the idea that leukemia is a neoplastic disease. Transplantation studies indicate that mouse leukemia behaves in general as do the other neoplasms of this species.[13, 23]

In these experiments treatment with leukemia-inciting agents was begun when mice were 10 weeks of age. Susceptibility to at least two of these agents (carcinogenic hydrocarbons and x-rays) was greater if administration was

begun earlier in life.[19, 31] It is likely that the incidence of induced disease would be higher in most stocks if experiments were begun with younger mice. It is also possible that if younger animals were used certain stocks would prove not to be completely refractory to any agent. Under the conditions of these experiments, however, it is possible to make the comparison of leukemogenic effectiveness set forth in Table IX.

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EVALUATION OF BONE MARROW CONCENTRATION TECHNIQUES

A Modified Method for the Simultaneous Preparation and Staining of Blood and Bone Marrow Films

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CORRELATION studies of the peripheral blood and bone marrow in diseases of the hemopoietic organs is a firmly established and universally recognized procedure. Sternal marrow aspiration allows the investigator to study the blood cells in their formative stage. This knowledge has given him a new perspective into the diagnosis and management of blood dyscrasias. Sternal aspiration is not the sole method or answer to hematologic diagnosis but it is important in the proper evaluation of abnormal blood findings. In many cases it is the key to the diagnosis. The study of an abnormal blood picture is not complete without an analysis of the sternal marrow.

The two methods of sternal marrow biopsy in general use are the trephine and aspiration. Of these two methods sternal aspiration has many advantages; thus, it is the method of choice of most investigators. The one objection to this method is that along with aspiration of bone marrow cells one must of necessity withdraw peripheral blood (sinusoidal) at the same time. This may dilute the bone marrow so greatly that when one uses fixed films he may see only a few bone marrow cells on the film. This one objection has been overcome by the use of the concentration technique.

REVIEW OF THE METHODS USED FOR CONCENTRATING BONE MARROW CELLS

Several investigators have described methods for concentrating bone marrow cells. Reich[16] in 1935, by means of a specially constructed needle which must be driven into the sternum, aspirated 10 c.c. of bloody fluid. This material was mixed with 2 c.c. of 1.4 per cent sodium oxalate solution and centrifuged, and films were made of the buffy layer. Recently, Reich[17] has modified his original method by aspirating 4 c.c. of marrow fluid and concentrating the buffy layer a second time in a small tube. In 1937 Vogel and associates,[21] in some of their bone marrow studies, aspirated 2 c.c. of marrow fluid and after using one drop of 30 per cent sodium oxalate as an anticoagulant, placed some of the marrow material in a Wintrobe tube and centrifuged it for five to ten minutes. In this way a relatively accurate red cell, white cell, and platelet volume was determined. Occasionally a fat layer was seen at the top of the tube. The white cell layer was carefully removed with a Wright capillary pipette and mixed to insure uniformity, and films were made. The disad-

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Aided by a grant from Armour and Company.

vantages of the methods used by Reich and Vogel and associates are, first, that the large amount of marrow blood aspirated does not lend itself to accurate quantitative study in the hematocrit tube and, second, that the distortion of cell morphology, especially that of the nucleus, due to oxalates may be misleading. This may account for the number of unclassified cells recorded by some workers. Schleicher and Sharp[18] in 1937 described a method of preparing and staining bone marrow in which heparin was used as an anticoagulant. Approximately 0.5 c.c. of marrow fluid was aspirated through a specially devised sternal needle (Klima and Rosegger type[9]) and placed into a small paraffin-lined test tube containing a sufficient amount of heparin to prevent coagulation. The heparinized marrow fluid was pipetted into a Wintrobe hematocrit tube and centrifuged at high speed for five minutes. The white myeloid layer in the hematocrit tube, plus an equal column of red cells (peripheral blood), was mixed with a volume of plasma equal to both of the cellular layers. The mixture of bone marrow cells, red blood cells, and plasma was mixed in a paraffin-lined watch glass, and margin-free films were made with a cover slip. The films were stained with May-Grünwald-Giemsa combination. The disadvantages of this method are, first, that the small amount of marrow fluid aspirated (0.5 c.c.) does not lend itself to accurate quantitative studies in the Wintrobe hematocrit tube and, second, that the dilution of bone marrow cells by the addition of the column of red blood cells (peripheral blood) defeats the purpose of concentrating marrow blood. In the method of Schleicher and Sharp the dilution factor is directly related to the quantity of the white myeloid layer; that is, the higher the column of bone marrow cells the greater the dilution with peripheral blood (column of red cells). A. S. Gordon[4] in 1939 used post-mortem bone marrow material for the quantitative estimation of the cellular elements of the bone marrow and for differential studies. H. Gordon[5] in 1941 described a method for preparing films and sections of the marrow fluid in which dry oxalate was used to prevent clotting of the marrow fluid. Accurate cytologic identification is difficult in post-mortem material, and the changes become more pronounced the longer after death one waits to study the bone marrow cells. The effect of oxalates on cytology of bone marrow cells has been discussed elsewhere.[10]

AUTHOR'S METHOD OF OBTAINING AND CONCENTRATING BONE MARROW CELLS

The method of sternal aspiration and preparation of marrow specimens with a detailed description of the apparatus (Fig. 1) and procedure was first reported by the author[10] in 1939. To state it briefly: Under aseptic technique and procaine anesthesia the sternum (midline) is punctured in the second or third interspace, preferably the former site, with a specially devised 16 gauge needle.* This needle is one devised by Klima and Rosegger modified by Poncher and the author in 1937 for a Luer syringe attachment; thus, an adapter, as required for the original needle, is not necessary. The needle is forced into the bone perpendicular to the sternum until a sudden "give" indicates that the marrow cavity has been reached (Fig. 2). The stylet is then removed. With a tight fitting, dry 5 or 10 c.c.

*University of Illinois Sternal Needle, made by V. Muller and Company, Chicago, Ill. Although a guard is a part of the needle, from a practical point of view it is of little help in gauging the thickness of the anterior plate of the sternum. The guard serves its greatest usefulness by allowing one to obtain a firm grip of the needle while forcing it into the sternum. In obese individuals it may be necessary to remove the guard from the needle before attempting to enter the sternum.

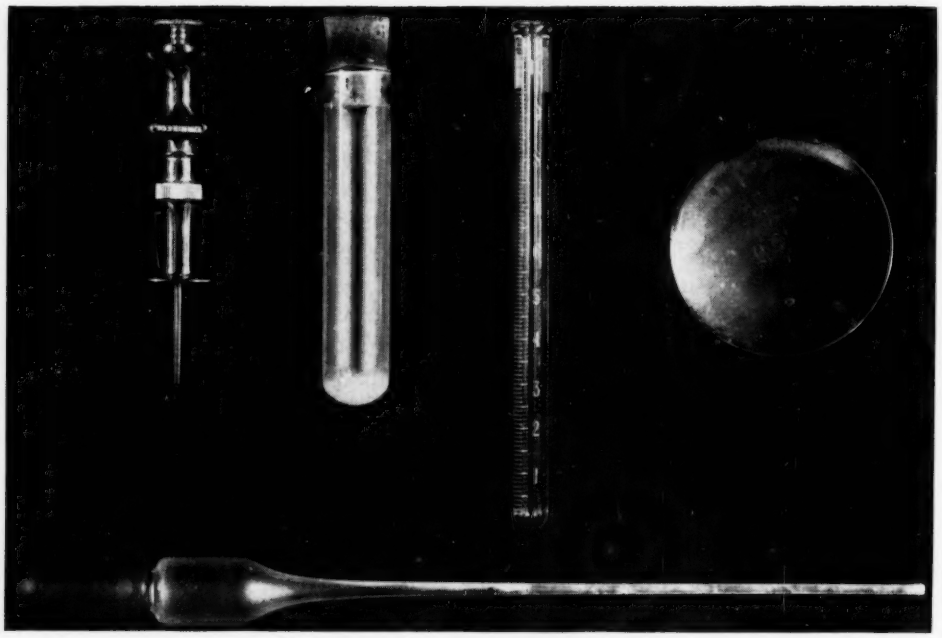

Fig. 1.—Apparatus for sternal puncture (concentration technique) showing the Klima and Rosegger sternal needle modified for Luer syringe attachment, paraffin-lined tube containing powdered heparin, Wintrobe hematocrit tube, paraffin-lined pipette, and watch glass.

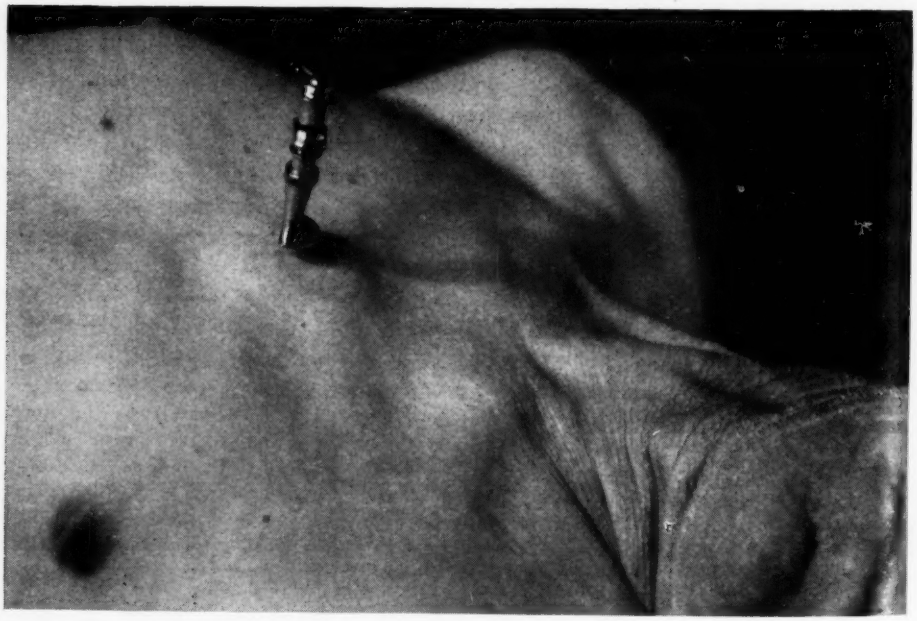

Fig. 2.—Sternal needle in position in the sternum at the second costal interspace. Note that the sternal needle in situ should be perpendicular to the sternum.

syringe, exactly 1 c.c. of marrow fluid is aspirated and immediately placed in a paraffin-lined tube containing a minute amount of heparin.* The tube is gently inverted several times to insure the anticoagulant effect of the heparin. Usually, if a successful sample of bone marrow has been obtained, a more or less dense suspension of small tissue particles will be visible.[10] The 1 c.c. of heparinized material consisting of peripheral blood (sinusoidal), hemopoietic marrow, and fat is pipetted into a Wintrobe hematocrit tube and centrifuged for approximately five minutes at about 2,000 r.p.m. Centrifugation separates the following layers reading from top down in the tube (Fig. 3, *A*): (1) fat, yellow and

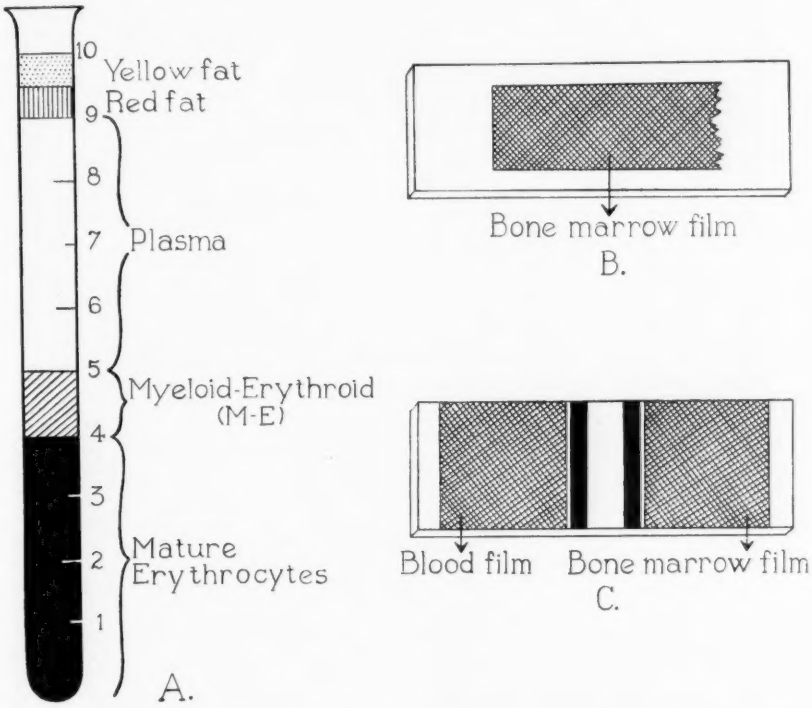

Fig. 3.—*A*, Hematocrit tube illustrating the composition of the several layers (volumetric) of the bone marrow material after centrifugation. *B*, Diagrammatic representation of the conventional bone marrow spread. The margin-free bone marrow film spread is made with an 18 by 22 mm. rectangular cover glass (same thickness as the counting chamber cover glass). *C*, Diagrammatic representation of a peripheral blood and bone marrow preparation with wax marking pencil lines on the inner edge of the film.

red, (2) plasma, (3) myeloid-erythroid cells; this layer also contains the megakaryocytes, and (4) erythrocytes from bone marrow sinuses. The heights of the several layers are recorded.† The fat and most of the plasma are removed separately and discarded. The myeloid-erythroid layer, together with a small amount of plasma, is pipetted off separately, transferred to a paraffin-lined watch glass, mixed, and from this material, films and cell counts are made. This method has been followed by the author and his associates in more than 3,385 practically simultaneous studies of the bone marrow and peripheral blood.[11-14]

*Powdered Heparin, Lot No. 153, Hyson, Westcott, and Dunning, Baltimore, Md. This type of heparin is inexpensive and 1.0 Gm. will suffice for approximately 350 to 400 sternal punctures.

†Normal average quantitative values for adults as represented in the hematocrit tube are as follows: Fat (yellow and red), 3.2 per cent; plasma, 45 per cent; myeloid-erythroid (M-E), 6.8 per cent; and erythrocytes, 45 per cent. Before any definite figures can be accepted, a large number of cases from all age groups are needed where quantitative studies are made from marrow obtained under standardized techniques. Of these values the myeloid-erythroid is of hematologic significance.

In the original method a small drop of marrow fluid is transferred to a new and perfectly clean microslide and with an 18 by 22 mm. rectangular cover glass a margin-free film is made (Fig. 3, *B*). The film is stained with Wright's stain or May-Grünwald-Giemsa dyes, except that a double concentration of Giemsa stain as recommended by Ferrata[3] and Downey[2] is used. A control film that has been made from a drop of nonheparinized marrow fluid is also prepared in the same way.

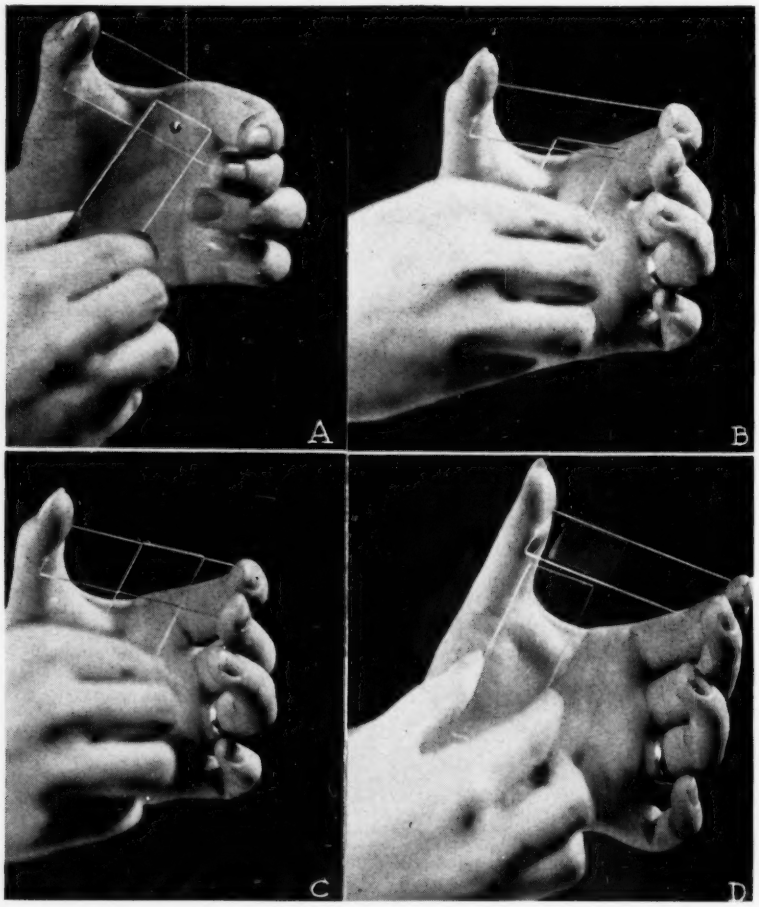

Fig. 4.—The technique of making blood and bone marrow films on one microslide. *A* to *D* show the technique of making the blood film. The same technique is followed for making the bone marrow film except that the opposite edge of the microslide is used.

Preparation of Marrow-Blood Films by Double Slide Technique.—In order to utilize a microslide for films of both blood and bone marrow, the following technique is used: Place a small drop of concentrated marrow fluid on the near lower edge of a scrupulously clean standard 3 by 1 inches microslide approximately one-quarter of an inch from one end of the slide. With a second slide, one end of which is used as a spreader and held at an angle of 30 to 45 degrees (Fig. 4), touch the second slide to the first slide so that the drop of blood is within the angle thus formed. Draw the second slide toward the drop of blood until the end of the slide just touches the drop and the blood spreads along the acute angle by capillarity. A moment is allowed for this to occur, after which both slides are opposed and the blood "sandwiched" and spread between them. The slides are then immediately and quickly drawn apart. The first slide which contains the usable film of the bone marrow

is finally dried by air in motion.* On the opposite end of the same slide place a drop of blood from the finger or ear and repeat the procedure as described for making the bone marrow film. This bone marrow-blood microslide preparation is now ready for staining. Before staining, a thick line by means of a marking pencil is drawn along the inner edge of both the bone marrow and blood films (Fig. 3, *C*).† The marking pencil line serves to prevent the stain from overflowing and prematurely staining the blood film on the opposite end of the slide. Note that the bone marrow and blood films are spread along the width of the microslide rather than the conventional length of the slide. A good bone marrow

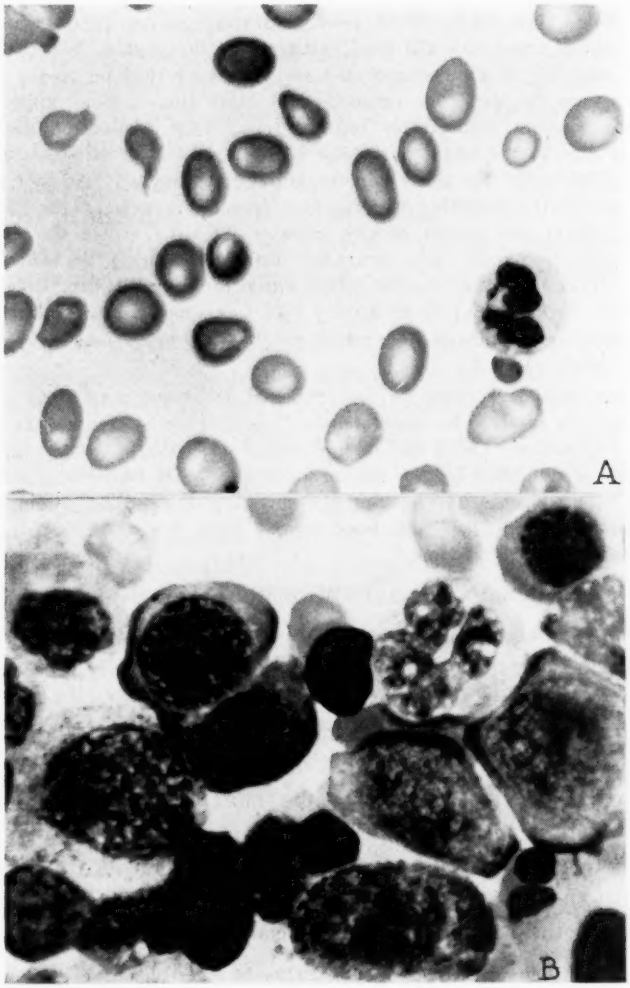

Fig. 5.—Photomicrograph of the (*A*) peripheral blood and (*B*) bone marrow (megaloblastic erythropoiesis) from a patient with pernicious anemia made from a peripheral blood and bone marrow preparation as illustrated in Fig. 3, *C*.

*This method was first used by Dr. Raphael Isaacs for the preparation of either blood or bone marrow films.

†When May-Grünwald-Giemsa dyes or Wright-Giemsa stains are used for staining the bone marrow-blood preparation, one can dispense with the marking pencil line. Also, when slight overstaining of the blood film square does not interfere with morphology details, the marking pencil line that separates the blood and bone marrow films can be dispensed with and the entire preparation stained as follows: Allow the Wright's stain to act for three minutes; then dilute with as much distilled water as the slide will hold, mixing very thoroughly. Allow this to stand fifteen to twenty minutes, and then wash with distilled water and air-dry. Prolonged washing in distilled water will usually take care of the overstaining of the blood.

and peripheral blood preparation should be smooth, homogeneous, and have even edges. The films of the bone marrow and peripheral blood should occupy approximately the opposite one-third of the end of the slide, as seen in Fig. 3, *C*. The area between the inner edges of the bone marrow and blood films, after the preparation has been stained and the marking pencil marks have been wiped away, is sufficient space for ordinary labeling.

Staining of Bone Marrow and Peripheral Blood Preparation.—For general routine work, Wright's stain is most satisfactory.* The preparation is placed on a staining rack and the bone marrow film is stained first. The "square" with the bone marrow film is covered with Wright's stain and allowed to act for three minutes, this procedure serving to fix the smear. The stain is then diluted with distilled water,† as much as the lower end of the slide containing the marrow film will hold, mixing very thoroughly; the marking pencil line along the inner edge of the film prevents the stain from overflowing to the opposite side of the slide and staining the blood film prematurely. Allow this to stand approximately fifteen minutes; without washing immediately begin staining that portion of the slide with the peripheral blood film in the usual way that is, Wright's stain one and one-half minutes followed by distilled water for three minutes, mixing thoroughly. At this point the entire slide may become diluted following the overflow from both ends of the slide. The entire staining period should not exceed twenty minutes. Finally, rinse the entire slide with distilled water until the water runs, returning clear while tilting the slide. Wipe off the marking pencil marks and rinse with distilled water for a second time, place in a vertical position in a slide rack, and allow to air-dry. If this procedure is followed there will be obtained well-tinted bone marrow and peripheral blood preparations with no film or precipitated stain visible (Fig. 5).

Preparations made according to concentration technique give bone marrow patterns comparable in certain respects to those seen in bone marrow sections; thus, they are called "tissue-like" preparations. With the oil immersion objective, a differential count may be performed. This is simplified by the use of an ocular which contains a Whipple grid[10] to divide the field into segments. By moving the stage one can readily examine the peripheral blood; thus, the peripheral blood and bone marrow films are available for examination on the same microslide.

COMMENTS

The author's method of concentrating and preparing bone marrow has a number of advantages: (1) Myeloid-erythroid (M-E) hypoplasia and hyperplasia is relatively accurately measured; thus, quantitative changes (myeloid-erythroid volume in the hematocrit tube) as well as qualitative studies (differential counts of the bone marrow film) may be correlated. (2) The separation of the erythrocytes in the hematocrit tube, most of which are derived from the sinuses of the bone marrow, eliminates the admixture of peripheral blood that is responsible for the dilution of the bone marrow elements. (3) Fat which interferes with the proper staining of the bone marrow cells is removed by the centrifugation of the marrow fluid. It also gives a relatively accurate estima-

*Wright's stain is prepared by dropping 1 Gm. of certified Wright powder on the surface of 600 c.c. of chemically pure (acetone-free) absolute methyl alcohol in a ball mill containing one charge of pebbles. The ball mill is allowed to rotate in an electrically propelled machine overnight. The stain is stored in a tightly corked 800 c.c. dark brown bottle and allowed to stand about seven days. The stain is filtered into a 60 c.c. dispensing bottle and kept tightly corked when not in use. If a ball mill is not available, grind 0.1 Gm. of Wright powder with a few cubic centimeters of the alcohol in a mortar. Slowly add the alcohol, a few cubic centimeters at a time, until 60 c.c. of alcohol is added; then grind thoroughly. This process should take from twenty to thirty minutes. All the stain must go into solution. If possible, allow to stand one to two days. Filter before use.

†We have found from experience that double or triple distilled water gives better and more constant results than does a buffer solution of pH 6.4. Much of the cause of poor staining is due to the use of unclean slides or to poorly prepared films. A five gallon bottle of distilled water with an attached siphon is used for diluting Wright's stain and washing off the stain. The use of this water for as long as three months gives uniformly well-stained and well-tinted films.

tion of the amount of fat in the bone marrow specimen and is of value in the study of certain diseases. (4) The method lends itself to the preparation of a large collection of uniformly prepared and stained films for teaching classes in clinical pathology and hematology. The preparation of the blood film on the same microslide containing the marrow film is a practical method for the simultaneous study of hematologic material. It is an ideal method of filing both blood and bone marrow films for future reference.

One of the routine methods followed at the United States Naval Medical School[15] for preparing the aspirated marrow blood is to concentrate bone marrow using an ammonium-potassium oxalate mixture* as an anticoagulant. Although this oxalate mixture causes little if any distortion of cell morphology, a control marrow film should be prepared from a drop of the marrow fluid before it is added to the anticoagulant.

A number of investigators, including Jones,[8] Hebbel,[6] Holly,[7] Stasney and Pizzolato,[20] and Schleicher,[19] have used the author's concentration technique for the quantitative and qualitative studies of the bone marrow in normal and pathologic conditions.

The histologic study of the bone marrow can be provided by the use of marrow particles either prior to or following heparinization and concentration of the marrow fluid. These are placed in Zenker-Formalin solution and the sections after fixation are prepared in the usual way with paraffin and stained with hematoxylin-eosin or Giemsa. Recently, Berman and Axelrod[1] have described a method of utilizing bone marrow aspiration material for volumetric readings, smears, imprints, and histopathologic sections. The sections are utilized in studying the topographic relationship of the bone marrow. Frequently these hemopoietic particles are too small to be of value. This is especially true in hypoplastic bone marrows. Then again detailed and accurate cell morphology and identification is not well preserved in sectioned material. Marrow blood that has been allowed to clot and treated as described for making histologic preparations is of little value for the study of bone marrow. Also, anyone whose experience with sternal aspiration has been at all intensive has observed cases in which it has been impossible to obtain bone marrow material for study, the so-called dry tap. This is observed in cases of myelosclerosis and myelofibrosis. Under such circumstances it is more appropriate to use a small trephine and remove a small plug of bone marrow. The bone marrow plug can also be used for making smears and imprint (abklatsch) preparations of the bone marrow.

Clinical hematology may derive a great deal of benefit from the study of the peripheral blood and bone marrow. However, it cannot be too strongly emphasized that data obtained by a study of all hematologic aspects of a case should never be sufficient to make a diagnosis. The clinical picture obtained by a complete history and thorough physical examination still remains the most important factor in all hematologic procedures and investigations.

*Ammonium-potassium oxalate mixture is prepared as follows: 1.2 Gm. of ammonium oxalate and 0.8 Gm. of potassium oxalate are dissolved in 100 c.c. of distilled water. Place 0.1 c.c. of this mixture in a small test tube and evaporate to dryness. This amount of anticoagulant suffices to prevent the clotting of about 1 c.c. of aspirated bone marrow fluid.

CONCLUSIONS

1. The advantages and disadvantages of bone marrow concentration techniques in general use for the preparation and studying of bone marrow are discussed.

2. A method is described which utilizes a microslide for the uniform preparation and staining of both peripheral blood and bone marrow films.

3. The advantages of the author's concentration technique for the study of bone marrow are discussed.

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QUANTITATIVE CYTOLOGIC STUDY OF MULTIPLE STERNAL MARROW SAMPLES TAKEN SIMULTANEOUSLY

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PREVIOUS qualitative and quantitative studies of the cytologic constituents of the bone marrow taken from widely separated portions of the marrow lead to the conclusion that there probably is a relative identity in the cellular organization of the red marrow throughout the body. Our former study was based on preparations obtained from fourteen persons after their accidental death.[1] The samples were obtained from three different regions shortly after death, and imprint preparations were made. In experimental work marrow samples were obtained from different portions of the marrow soon after the animal had been sacrificed.[2-6] With this technique we found that cytologic appraisal of any region of marrow will reveal, within the limits of error, what is the trend of the marrow changes elsewhere in the body.

Recently, several authors[7-9] found differences in the cellular composition of the different portions of the marrow, claiming that the marrow is not a homogenous structure. Reich and Kolb[10] reported a quantitative study on a series of multiple sternal marrow samples, taken simultaneously, and concluded that quantitative determinations are inaccurate.

To ascertain the basically important point whether one marrow sample would give a representative picture of the bone marrow function in the present study, we examined quantitatively and qualitatively two portions of the sternal marrow by obtaining samples from two different sites simultaneously.

MATERIALS AND METHODS

Thirty patients with a wide variety of pathologic conditions at the Charity Hospital of Louisiana were used in this study. The marrow was aspirated through an 18 gauge spinal needle from two sites of the body of the sternum, about 2 or 3 cm. apart. Care was used to remove not more than 1 c.c. of liquid. The marrow upon removal was gently expressed through the needle into a bottle containing 2 mg. of ammonium potassium oxalate. From the material remaining in the needle, cover-slip preparations were made and rapidly dried by waving through the air; these were stained with Wright-Giemsa stain after several hours of additional drying.

From the oxalated marrow the total nucleated elements were counted employing white cell pipettes and diluting with 5 per cent acetic acid. The counts were done on the regular Levy counting chamber using 5 to 25 squares of the large central square, depending on the number of cells present. The following formula was used:

$$\text{Total nucleated elements} = \frac{\text{Number of cells counted} \times \text{dilution}}{\text{Volume of chamber}}$$

The unused marrow was placed in a Wintrobe tube and centrifugalized at 2,000 r.p.m. for five minutes and the percentage of the marrow fractions was tabulated according to the method of Limarzi.[11] With this technique, 5 to 8 per cent were found as normal values for

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†From Jefferson Medical College.

TABLE

CASE	ASPIRATION SITE	RACE	SEX	AGE	DIAGNOSIS	PERCENTAGE OF FRACTION OF MARROW				TOTAL NUCLEATED ELEMENTS PER THOUSAND	RETICULO-ENDOTHELIAL CELL	MYELOBLAST	LEUKOBLAST	PROMYELOCYTE	MYELOCYTE	METAMYELOCYTE	NEUTROPHILE	EOSINOPHILIC MYELOCYTE	EOSINOPHILIC METAMYELOCYTE	EOSINOPHILE	BASOPHILIC MYELOCYTE	BASOPHILIC METAMYELOCYTE
						FAT	PLASMA	MARROW	HEMATOCRIT													
1	1	C	M	50	Peptic ulcer	4.5	52.5	10.0	33.0	117.1	0.2	0.4	0.8	1.4	5.6	9.4	16.6	--	--	0.4	0.2	0.2
	2					4.0	55.0	8.0	33.0	68.2	0.2	0.4	0.2	0.6	2.6	9.4	17.0	--	--	1.0	--	--
2	1	C	M	50	Carcinoma of stomach	3.0	51.0	3.0	43.0	20.3	0.4	1.2	1.8	2.6	7.8	9.4	23.6	0.8	0.2	2.4	0.2	0.2
	2					1.5	53.5	2.0	43.0	17.8	0.2	0.8	1.4	3.0	10.6	12.4	23.8	0.2	0.4	2.6	--	--
3	1	C	F	53	Pernicious anemia	--	68.1	6.6	23.3	28.6	--	0.8	3.2	10.6	18.6	9.8	13.3	0.2	0.4	1.4	0.2	--
	2					--	69.5	4.5	26.0	22.0	0.6	2.0	4.4	15.2	16.4	6.0	7.6	0.4	0.6	2.4	0.6	0.2
4	1	C	F	71	Pernicious anemia	2.0	73.3	7.7	17.0	55.0	0.8	0.2	1.8	3.6	8.6	6.0	23.6	0.2	0.2	0.4	0.4	0.4
	2					1.4	75.8	5.7	17.1	40.8	0.8	0.6	1.2	1.6	7.8	7.2	20.0	--	--	0.6	--	--
5	1	C	F	71	Pernicious anemia*	1.1	69.7	13.5	15.7	85.5	0.4	0.2	0.6	1.2	2.6	3.2	7.4	--	--	1.8	--	--
	2					0.7	75.3	4.5	19.5	49.5	1.0	0.6	1.0	4.6	5.2	5.0	12.8	--	--	1.2	--	0.2
6	1	W	M	55	Pernicious anemia†	--	54.0	8.0	38.0	71.3	0.2	0.2	1.4	1.8	9.0	15.6	22.6	0.4	1.2	2.6	--	--
	2					--	56.0	6.0	38.0	46.1	0.6	0.6	0.4	4.2	4.0	12.8	28.2	--	0.2	5.0	0.6	0.6
7	1	W	M	34	Pernicious anemia‡	2.5	43.8	12.3	41.4	113.5	--	1.0	2.0	3.8	11.4	15.6	13.6	1.4	2.2	2.2	--	--
	2					2.0	50.0	5.0	43.0	47.4	0.4	0.4	1.4	5.0	8.8	13.4	18.0	1.2	1.6	4.2	--	--
8	1	W	M	50	Macrocytic anemia; received folic acid	1.5	64.5	3.0	31.0	25.1	--	0.2	0.4	1.8	5.8	10.2	28.4	0.2	0.6	0.8	--	--
	2					5.2	59.1	7.1	28.6	78.8	0.4	1.0	1.6	3.2	7.8	10.6	21.8	--	0.2	1.6	0.2	0.2
9	1	W	F	25	Bleeding peptic ulcer	3.6	56.0	7.4	33.0	72.3	--	0.4	2.2	5.2	8.8	13.6	18.4	0.2	0.2	2.0	0.6	0.2
	2					2.3	58.4	7.6	31.7	84.3	0.4	0.2	2.6	2.4	7.6	18.2	22.6	0.2	0.4	2.4	--	0.2
10	1	C	M	18	Sickle cell anemia	--	56.8	17.0	26.2	201.0	0.4	2.2	0.4	1.0	2.4	5.2	6.2	0.2	--	1.0	--	--
	2					--	52.0	23.0	25.0	214.0	0.4	0.3	0.8	1.8	5.0	5.8	11.2	--	0.4	1.2	--	--

*After four days of liver treatment.
†After two weeks of liver treatment.
‡After one month of liver treatment.

bone marrow cells. We found the following distribution of the different fractions in the centrifugalized marrow in fourteen apparently healthy medical students, ten men and four women: fat, 1 to 3 per cent; plasma, 40 to 54; marrow, 3 to 11; and hematocrit, 40 to 51. The total number of nucleated elements was 43,000 to 105,000 per cubic millimeter. In the differential counts, a minimum of 500 cells was counted from each marrow sample. The damaged cells were counted also when encountered associated with well-preserved cells. The percentage distribution of all cells observed and the myeloid-erythroid ratio of each sample were computed and tabulated.

COMMENT

The numerical data are tabulated in Tables I to III.

In Table I, data for anemias and conditions leading to anemia are tabulated. Cases 1, 5, and 10 exhibit marked proliferation of erythroid cells with inversion of the myeloid-erythroid ratio. The values of these ratios in the two samples obtained from two sites of the sternum reveal differences but express a similar trend of changes.

TABLE I

BASOPHILIC METAMYELOCYTE	BASOPHILE	MONOCYTE	LYMPHOCYTE	PLASMA CELL	PRONORMOBLAST	BASOPHILIC NORMOBLAST	POLYCHROMATIC NORMOBLAST	ORTHOCHROMATIC NORMOBLAST	NORMOBLAST IN MITOSIS	MYELOID CELL IN MITOSIS	DAMAGED CELL	MEGAKARYOCYTE	IMMATURE LYMPHOCYTE	DAMAGED LYMPHOCYTE	HETEROPHILE	PROMEGALOBLAST	BASOPHILIC MEGALOBLAST	POLYCHROMATIC MEGALOBLAST	ORTHOCHROMATIC MEGALOBLAST	MYELOID-ERYTHROID RATIO
0.2	--	0.2	12.0	1.2	1.0	1.4	8.2	32.4	0.8	0.2	5.8	0.8	0.6	--	0.2	--	--	--	--	0.8
--	0.2	0.4	12.8	1.0	1.4	3.8	12.2	24.8	0.6	--	10.2	0.4	0.6	--	0.2	--	--	--	--	0.8
0.2	--	1.6	20.4	0.4	0.2	1.2	3.4	9.8	0.4	0.2	11.0	--	0.4	--	0.6	--	--	--	--	3.4
0.2	--	0.4	16.2	0.4	1.0	0.6	2.4	6.2	0.2	0.3	16.4	--	--	--	0.2	--	--	--	--	2.1
--	--	--	11.8	--	2.0	0.8	0.6	8.0	0.4	0.4	13.4	--	--	--	0.6	0.2	0.4	1.0	2.0	3.8
0.2	0.4	--	15.0	2.2	1.6	1.0	0.8	8.4	0.2	--	9.2	--	--	--	0.6	0.2	0.6	2.2	1.2	3.5
0.4	--	0.2	10.6	--	3.6	4.2	3.8	3.4	1.0	0.2	16.0	0.2	0.4	--	0.4	4.2	2.4	1.8	1.4	1.8
--	--	--	16.4	0.2	2.2	4.4	4.4	6.8	0.6	--	14.2	--	--	--	--	5.4	2.4	2.2	1.0	1.3
--	--	--	7.2	--	--	2.6	42.2	4.6	0.2	0.2	25.0	0.4	--	--	--	--	--	--	--	0.4
--	0.2	--	8.2	0.2	1.2	1.6	42.2	8.2	0.8	0.4	8.2	--	0.2	--	0.2	--	--	--	--	0.5
--	0.4	--	12.6	--	0.4	2.8	1.6	21.4	0.4	--	3.0	--	1.0	--	1.0	--	--	--	--	1.9
0.6	0.8	0.6	19.8	0.4	0.8	0.6	1.6	10.4	0.2	--	6.0	0.2	0.2	--	1.6	--	--	--	--	4.3
--	0.4	--	9.6	--	0.8	1.4	2.6	17.6	0.2	0.2	11.0	--	1.8	--	0.6	--	--	--	--	2 4
--	0.6	0.6	10.4	0.4	0.4	1.4	2.2	12.8	0.4	0.2	14.4	--	0.3	--	0.2	--	--	--	--	3.4
0.2	0.8	2.2	9.8	--	0.4	1.6	6.4	16.8	0.4	--	12.6	--	--	--	0.4	--	--	--	--	2.0
0.2	0.2	0.8	12.4	--	0.8	2.2	8.0	16.2	1.4	--	8.0	0.2	0.4	--	0.4	--	--	--	--	1.7
0.6	0.2	0.2	18.8	1.0	0.2	1.0	4.8	11.6	1.0	--	6.2	--	1.6	--	1.4	--	--	--	--	2.9
--	0.2	0.2	20.6	0.2	0.4	0.6	3.4	10.6	--	--	5.8	0.2	--	--	0.8	--	--	--	--	3.9
--	--	--	11.6	0.8	3.2	8.2	12.4	31.8	1.4	--	10.4	0.4	--	--	0.6	--	--	--	--	0.4
--	--	--	11.0	0.4	1.6	5.6	11.4	36.2	1.4	0.2	4.0	--	0.2	--	--	--	--	--	--	0.5

Illustrated in Table II are cases of leukemic conditions with strikingly similar proliferation of the pathologic cells in the two samples. The case of idiopathic purpura in both samples shows relatively normal values.

In Table III are included, among others, cases with tendencies to form localized, nodular changes in the red marrow, like multiple myeloma and Boeck's sarcoidosis. The values for the myeloid-erythroid ratio, not including the pathologically proliferating cells, again reveal parallel changes in the two samples.

All the cases included were of different sex, age, and race with wide variety of pathologic conditions. Because of these great differences between the examined cases, no statistical analysis was attempted. A comparison between the cellularity of the two samples from the same sternum disclosed variations which reflected in the quantity of the different fraction of the marrow as well as in the total number of nucleated elements and in the percentage distribution of different cells.

The differences in the quantitative data may be caused by the technique of obtaining the samples. Dameshek and associates[12] pointed out that the tech-

TABLE II

CASE	ASPIRATION SITE	RACE	SEX	AGE	DIAGNOSIS	PERCENTAGE OF FRACTION OF MARROW: FAT	PLASMA	MARROW	HEMATOCRIT	TOTAL NUCLEATED ELEMENTS PER THOUSAND	RETICULO-ENDOTHELIAL CELL	MYELOBLAST	LEUKOBLAST	PROMYELOCYTE	MYELOCYTE	METAMYELOCYTE	NEUTROPHILE	EOSINOPHILIC MYELOCYTE	EOSINOPHILIC METAMYELOCYTE	EOSINOPHILE	BASOPHILIC MYELOCYTE	BASOPHILIC METAMYELOCYTE
11	1	W	M	35	Leukopenia	0.6	52.3	4.7	42.4	10.6	0.6	0.2	0.6	1.6	4.2	12.4	37.6	--	0.2	3.2	0.2	0.2
	2					0.7	51.2	4.6	43.5	43.6	0.6	0.6	1.0	2.8	7.2	15.2	31.6	0.2	0.2	1.8	0.4	--
12	1	C	M	53	Chronic lymphatic leukemia			Insufficient			0.2	--	--	--	0.6	0.6	1.0	--	--	--	--	--
	2							Insufficient			--	--	--	--	--	0.8	0.4	--	--	0.2	--	--
13	1	C	M	40	Fever (?)	1.0	55.0	12.0	32.0	71.6	0.4	0.4	1.6	3.2	7.8	21.2	25.8	0.2	0.2	2.6	--	--
	2					1.7	52.5	10.0	35.8	35.5	--	0.2	1.0	4.4	9.0	22.2	20.8	--	0.6	0.4	--	0.4
14	1	W	M	35	Idiopathic purpura	1.0	57.0	3.0	39.0	23.3	0.2	0.4	0.8	4.8	9.8	19.6	20.0	0.4	1.0	--	--	--
	2					4.7	56.3	5.7	33.3	25.3	--	0.6	0.4	4.4	7.2	16.2	28.6	0.4	0.2	0.8	--	--
15	1	W	M	59	Polycythemia vera	0.7	37.6	1.7	60.0	16.2	--	0.4	0.6	2.6	9.0	7.8	29.0	--	--	1.0	0.8	--
	2					0.7	43.2	2.4	53.7	18.2	--	0.2	0.6	1.8	5.0	5.8	34.0	0.2	--	2.4	0.4	--
16	1	C	F	20	Stem cell leukemia	--	46.8	6.6	46.6	91.5	0.2	--	0.2	0.8	5.2	4.0	7.2	--	--	--	--	--
	2					--	39.0	11.0	50.0	105.4	0.8	--	0.8	1.4	2.8	2.0	5.8	--	--	--	--	--
17	1	C	M	69	Stem cell leukemia	--	45.0	23.0	32.0	147.0	--	--	--	--	0.4	--	4.8	--	--	0.6	--	--
	2					--	46.0	22.0	32.0	154.0	0.2	--	--	--	--	0.6	2.4	--	0.2	--	--	0.2
18	1	W	M	6	Stem cell leukemia	--	68.7	2.3	29.0	43.5	0.4	--	--	--	--	--	0.6	--	--	0.8	--	--
	2							Insufficient		153.7	0.4	--	--	--	0.4	0.2	0.2	--	0.2	--	--	--
19	1	W	M	64	Aleukemic myeloid leukemia	10.0	48.0	10.0	32.0	96.8	--	0.4	0.2	1.8	4.4	11.6	31.8	--	0.8	3.6	--	--
	2					33.5	28.5	21.5	16.5	308.0	0.4	0.6	1.8	4.4	13.2	23.2	23.4	0.4	0.2	2.0	--	0.2
20	1	W	M	35	Aleukemic myeloid leukemia	--	69.0	22.0	9.0	218.0	0.6	1.2	4.2	7.8	20.6	28.2	9.8	0.2	0.6	1.4	--	--
	2					--	75.0	15.0	10.0	209.8	0.2	1.0	5.4	7.6	16.8	22.8	10.8	0.2	0.2	0.2	0.2	0.8
21	1	W	M	32	Lymphosarcoma	1.7	50.0	0.8	47.5	7.5	0.4	--	0.6	2.0	3.0	4.6	19.6	--	0.2	1.4	0.2	0.2
	2					1.3	53.6	0.6	44.5	6.5	0.6	--	0.4	2.2	4.6	11.6	30.2	--	--	2.8	--	0.2

nique used for obtaining marrow samples was itself a possible source for differences in quantitative values of different cells. They compared the results of marrow samples obtained through biopsies and by punctures. In the latter, the more immature cells and the fixed cells were less numerous than in samples obtained through biopsies. It is possible that the suction force applied at puncture draws back more readily the free cells than the more immature cells, which according to Isaac's conception[13] are probably surrounded by firmer gelatinous substance than the mature cells. These marrow cells were enmeshed by a fine network of the fixed reticulum cells.

Another possible cause for the numerical differences is the unavoidable admixture with red blood cells. The larger the amount withdrawn, the more likely it is that the walls of sinusoids will rupture with resultant admixture with blood. Occasionally, even after withdrawing a small amount of fluid (0.2 c.c.), one may obtain pure blood with apparently no marrow cells present. It is possible that one aspirates the content of a sinusoid.

TABLE II

BASOPHILIC METAMYELOCYTE	BASOPHILE	MONOCYTE	LYMPHOCYTE	PLASMA CELL	PRONORMOBLAST	BASOPHILIC NORMOBLAST	POLYCHROMATIC NORMOBLAST	ORTHOCHROMATIC NORMOBLAST	NORMOBLAST IN MITOSIS	MYELOID CELL IN MITOSIS	DAMAGED CELL	MEGAKARYOCYTE	IMMATURE LYMPHOCYTE	DAMAGED LYMPHOCYTE	HETEROPHILE	STEM CELL	MYELOID-ERYTHROID RATIO	
0.2	--	2.8	19.0	0.2	0.2	0.6	4.8	4.2	0.2	--	5.8	--	--	--	1.2	--	6.2	
--	--	1.0	15.8	0.6	0.6	0.4	3.8	5.8	0.4	--	7.2	0.2	--	--	1.4	--	5.7	
--	--	--	37.0	--	--	--	0.2	0.2	--									
--	--	--	40.0	--	--	--	1.8	2.2	--	--	--	--	--	54.6	--	--		Preponderance of lymphocytes
										--	--	--	0.2	60.0	--	--		
--	--	0.2	7.4	1.2	0.8	3.0	7.0	2.2	--	--	14.4	--	--	--	0.4	--	4.9	
0.4	0.2	0.4	9.6	1.2	0.2	1.0	5.4	4.8	--	--	17.2	--	--	--	1.0	--	5.3	
--	--	1.2	12.8	0.4	0.6	2.6	10.0	3.8	0.6	--	11.0	--	--	--	--	--	3.2	
--	0.4	0.8	8.8	1.0	0.6	2.8	6.4	5.4	--	--	13.8	0.6	0.4	--	0.2	--	3.9	
--	--	1.0	22.4	0.4	0.2	1.6	3.2	3.2	0.2	--	11.2	0.2	--	--	4.0	--	6.6	
--	0.2	0.2	24.0	0.6	--	2.6	4.0	6.4	--	--	10.0	--	--	--	1.6	--	4.0	
--	--	--	7.8	0.8	--	0.2	0.6	0.6	--	--	6.2	--	--	--	--	66.2		Preponderance of stem cells
--	--	--	12.2	0.4	--	0.2	0.6	1.0	--	--	11.6	--	--	--	--	60.4		
--	--	--	2.2	--	0.2	0.4	0.8	3.2	--	0.8	12.4	--	--	--	--	74.2		Preponderance of stem cells
0.2	0.2	--	4.8	0.2	--	0.2	1.0	2.6	--	0.4	7.0	0.2	--	--	--	79.8		
--	--	--	15.0	--	--	0.2	0.2	0.2	--	0.2	17.4	--	--	--	--	65.0		Preponderance of stem cells
--	--	--	13.0	--	--	0.2	0.2	0.6	--	0.6	40.0	--	--	--	--	44.0		
--	--	0.6	12.6	0.2	0.4	1.8	4.0	10.4	0.6	--	14.4	0.2	0.2	--	--	--	3.2	
0.2	--	0.2	9.4	0.4	0.4	1.6	2.2	10.4	0.4	0.2	4.0	0.4	0.2	--	0.4	--	4.7	
--	--	--	5.6	--	0.4	0.4	2.6	9.4	--	0.8	5.4	--	0.2	--	0.6	--	5.8	
0.8	--	0.8	8.0	0.2	0.6	2.2	2.8	14.4	0.4	0.2	3.6	--	0.2	--	0.6	--	3.3	
0.2	0.4	0.8	23.2	2.4	0.2	0.2	1.6	2.4	--	--	35.6	0.2	0.2	--	0.6	--	7.5	
0.2	0.2	0.8	24.2	0.2	--	0.2	1.6	4.4	0.2	--	15.4	0.2	--	--	--	--	8.2	

In previous experimental work where the marrow samples from different bones were examined shortly after sacrificing the animals and no admixture of blood was possible, we found strikingly coordinated changes in different portions of the marrow examined.[2-6] Human marrow samples taken from different portions of the marrow shortly after death, without the possible admixture of blood, exhibited also a markedly similar cellular pattern.[1] In the enumeration of the total number of nucleated marrow cells, the clumping of the cells to each other or adherence of the cells to bony spiculae may produce discrepancies.

The third possibility for the numerous differences is the lack of homogeneity of the bone marrow. Custer[14] has shown that the cellular density of the marrow of the long bones may vary enormously in even closely adjacent regions and that the flat bones may be densely packed with cells, while the fatty marrow of the long bones may contain but few groups of cells with hematopoietic potency. Recently, Reiter[8] pointed out that there are irregular fatty areas present in the actively functioning red marrow of the sternum. Helpap,[7]

TABLE

CASE	ASPIRATION SITE	RACE	SEX	AGE	DIAGNOSIS	PERCENTAGE OF FRACTION OF MARROW				TOTAL NUCLEATED ELEMENTS PER THOUSAND	RETICULO-ENDOTHELIAL CELL	MYELOBLAST	LEUKOBLAST	PROMYELOCYTE	MYELOCYTE	METAMYELOCYTE	NEUTROPHILE	EOSINOPHILIC MYELOCYTE	EOSINOPHILIC METAMYELOCYTE
						FAT	PLASMA	MARROW	HEMATOCRIT										
22	1	C	M	18	Boeck's	0.5	51.5	6.0	42.0	61.5	--	--	0.4	2.2	9.2	15.8	26.6	--	0.6
	2				sarcoidosis	1.0	49.0	8.5	41.5	92.5	0.2	0.6	1.6	3.2	10.6	13.0	25.2	0.4	0.6
23	1	W	M	43	Multiple	1.2	40.4	2.4	56.0	17.0	--	0.2	0.6	3.2	4.4	11.2	31.6	--	0.2
	2				myeloma	1.0	39.0	2.0	58.0	16.3	--	0.4	1.6	3.4	6.8	14.2	28.4	0.2	0.6
24	1	W	M	43	Multiple	0.5	65.0	0.5	34.0	0.94	0.4	0.2	1.0	2.2	5.2	8.0	24.0	--	0.2
	2				myeloma	Insufficient				1.44	--	--	0.2	1.0	1.4	5.0	43.0	--	--
25	-	W	M	40	Fever (?)	0.7	60.6	2.7	36.0	16.8	--	--	0.6	3.4	6.2	10.2	30.4	0.2	0.6
	2					1.1	50.6	5.0	43.3	56.7	--	0.2	0.8	3.2	8.8	16.0	26.6	0.8	0.2
26	1	W	M	43	Thrombo-	1.0	63.5	4.5	31.0	55.5	1.8	1.4	1.4	2.8	9.2	10.2	16.4	0.2	0.6
	2				cytopenia	2.2	61.1	7.6	29.1	65.5	2.4	0.2	1.0	2.0	7.0	12.2	16.6	--	0.4
27	1	W	M	25	Hodgkin's	--	60.0	16.0	24.0	103.4	--	0.4	0.8	2.4	7.4	12.4	22.6	0.2	0.2
	2				disease	--	53.8	23.1	23.1	86.1	--	0.2	1.4	4.4	11.4	18.4	13.0	--	0.4
28	1	W	M	45	Cirrhosis	1.0	56.0	5.0	38.0	49.4	--	0.4	0.8	2.0	12.4	15.0	21.0	0.2	0.6
	2				of liver	3.0	52.0	10.0	35.0	82.7	--	0.2	0.8	0.6	8.2	16.8	23.8	--	0.2
29	1	W	F	54	Bone	3.0	46.0	7.5	43.5	81.6	0.2	0.2	1.2	2.0	6.6	14.0	15.2	--	0.6
	2				cyst	2.9	48.8	4.3	44.0	43.8	0.2	0.4	1.0	2.8	6.8	16.6	19.2	--	0.2
30	1	W	M	30	Lupus	6.4	51.4	4.3	31.9	40.2	--	0.2	0.8	2.6	6.6	16.2	24.0	0.6	1.4
	2				vulgaris	6.7	51.7	4.0	37.6	41.5	0.6	0.8	0.8	4.2	10.0	11.8	19.0	1.2	2.8

Reiter,[8] and Jeanneret[9] claimed differences in the cellular composition in different portions of the marrow.

The long bones of man and mammals usually contain yellow fatty marrow in which there are scattered centers or islands of hematopoietic cells. However, comparative studies of the bone marrow of flat bones and different portions of the long bones revealed the same trend in the relative percentage distribution of the hematopoietic cells in dogs and monkeys.[2, 3]

Our Cases 8, 14, and 19 illustrate clearly that probably there are irregular foci of fatty tissue in the red marrow also, with marked difference in fat content of simultaneously obtained sternal marrow samples. In spite of the numerous differences between the cells in the two simultaneously obtained marrow samples, the trend of the cellular differentiation is the same. This fact is illustrated in Tables I to III by the myeloid-erythroid ratios. If at one portion of the marrow the erythroid cells are proliferating, then another portion of the marrow will reveal similar erythroid stimulation. In stem cell leukemias, and in lymphatic leukemias there again was a remarkably similar increase of the abnormal cells encountered in the different samples. Even in the primary or secondary neoplastic processes of the marrow, with the formation of more or less circumscribed nodular areas, the uninvolved portions of the marrow reveal similar cellular population.

TABLE III

EOSINOPHILE	BASOPHILIC MYELOCYTE	BASOPHILIC METAMYELOCYTE	BASOPHILE	MONOCYTE	LYMPHOCYTE	PLASMA CELL	PRONORMOBLAST	BASOPHILIC NORMOBLAST	POLYCHROMATIC NORMOBLAST	ORTHOCHROMATIC NORMOBLAST	NORMOBLAST IN MITOSIS	MYELOID CELL IN MITOSIS	DAMAGED CELL	MEGAKARYOCYTE	IMMATURE LYMPHOCYTE	DAMAGED LYMPHOCYTE	HETEROPHILE	TUMOR CELL	MYELOID-ERYTHROID RATIO
0.8	0.2	--	--	1.8	13.4	1.0	0.4	1.0	4.4	11.0	0.2	--	10.6	0.4	--	--	--	--	3.3
1.0	--	0.4	--	1.4	12.6	0.4	--	0.6	4.2	12.6	0.2	--	11.0	0.2	--	--	--	--	3.2
2.2	0.2	0.2	0.2	1.0	19.2	0.2	0.2	1.2	5.0	5.8	0.2	--	12.2	--	0.6	--	0.2	--	4.4
0.2	--	--	--	1.4	17.8	0.8	0.2	1.2	6.0	7.4	0.4	--	8.4	--	0.4	--	0.2	--	3.7
--	--	--	--	1.0	14.6	0.6	1.0	3.2	6.2	17.2	0.2	--	14.0	--	0.8	--	--	--	1.5
--	--	--	0.4	1.2	22.2	6.2	0.6	0.8	2.2	5.6	0.4	--	9.6	--	--	--	0.2	--	5.3
1.4	--	0.2	--	1.4	25.4	0.4	--	0.6	1.0	9.2	--	--	7.8	0.2	0.4	--	0.4	--	4.9
1.6	--	0.2	0.2	0.4	21.2	--	0.2	1.0	1.8	7.0	--	--	8.8	--	1.0	--	--	--	5.9
1.8	0.8	0.2	0.6	--	17.2	1.6	1.0	3.4	6.4	11.8	--	--	10.4	0.4	--	--	0.4	--	2.1
1.8	0.4	0.2	--	0.2	19.2	2.0	0.2	0.8	4.0	14.8	--	--	13.4	0.8	0.2	--	0.2	--	2.2
1.0	--	--	0.2	--	20.2	0.4	--	0.8	1.2	7.6	--	--	12.0	0.4	--	--	0.2	9.6	5.0
2.2	--	--	--	0.2	13.4	2.0	--	0.2	1.2	9.4	--	--	11.0	0.6	0.2	--	0.2	10.4	4.8
5.6	0.8	0.2	0.2	0.6	9.4	--	0.2	2.4	3.8	11.6	0.8	--	8.4	0.2	0.4	--	2.4	--	3.3
1.4	--	0.2	0.2	0.2	11.4	--	0.6	3.6	4.4	16.8	--	--	7.0	--	0.2	--	2.6	--	2.2
1.8	--	--	0.2	0.6	21.6	0.6	1.0	0.6	3.8	15.8	0.6	--	12.4	0.6	0.2	--	0.2	--	2.0
1.6	--	0.2	--	0.2	19.8	0.2	0.4	0.6	1.8	21.2	0.2	0.2	6.4	--	--	--	--	--	2.0
4.4	--	--	0.2	0.4	11.4	0.8	--	0.4	4.8	13.4	--	--	10.6	--	0.2	--	1.0	--	3.1
3.6	--	0.6	--	0.6	10.8	0.4	--	0.6	4.6	15.2	0.2	--	11.0	0.2	0.4	--	0.6	--	2.7

Hyperplastic myeloid or erythroid processes, abnormal cell proliferation of acute or chronic leukemic cells will produce uniformly generalized changes in the marrow. Bone marrow punctures with negative results warrant further confirmation and exclusion of technical error.

SUMMARY

In a series of different pathologic conditions, two samples of human sternal marrow were aspirated simultaneously. A comparative analysis of the two samples included the percentage distribution of the different fractions of aspirated marrow, the total number of nucleated cells, percentage distribution of the different cells, and the myeloid-erythroid ratio which were computed and tabulated. There were numerous differences between the two samples, but the trend of cellular differentiation was similar as exhibited by the myeloid-erythroid ratios. The morphologic or pathologic appraisal of any one region of the marrow will usually reveal trends in other portions of marrow throughout the body.

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THE RETICULO-ENDOTHELIAL ORIGIN OF THE BONE MARROW PLASMA CELLS IN HYPERSENSITIVE STATES

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INTRODUCTION

SINCE its description by Cajal in 1890, the plasma cell has been observed by pathologists in the hematopoietic tissue and in the specific lesions of varying clinical conditions. Huebschmann (1913) studied the spleens of patients dying of various infectious diseases and observed a splenic plasmacytosis resulting from pneumococcic, staphylococcic, diphtheric, and typhus infections. He found little or no increase in splenic plasma cells in cases of fatal miliary tuberculosis, anthrax, or in a luetic stillborn—all overwhelming infections. He associated the plasma cell with the production of antibodies.

Perlzweig, Delrue, and Geschickter (1928) and others since then have associated multiple myeloma, which is apparently a malignancy of the bone marrow resulting in the accumulation of plasma cells, with hyperglobulinemia. Since that time a number of investigations have related the plasma cell to hyperglobulinemia in clinical disease: Osgood and Hunter (1934) in plasma cell leukemia, Markoff (1937) in serum sickness, and Bing and Plum in agranulocytosis (1937). Bing, in various acute and chronic infections, myelomatoses, and other diseases (1940), concluded that the plasma cell of reticulo-endothelial origin was the source of the blood globulin. Fleischhacker (1940 and 1941) made many observations on patients with hyperglobulinemia and found that the bone marrow revealed simultaneous plasmocytosis. His conclusions were similar to those of Bing. Gormsen and Heintzelmann (1941) reported studies of blood globulin and sternal marrow in patients with serum sickness; they found a distinct parallelism between increased marrow plasma cells and hyperglobulinemia and stated that their observations support Bing's theories. Taussig and Somogyi (1940) reported hyperglobulinemia in six cases of granuloma inguinale and stated that it is common in lymphogranuloma inguinale. The presence of plasma cells in the lesions of these two diseases is well known. Kagan (1943) has furnished additional clinical evidence of the relation of globulin level and plasmacytosis.

The relationship of plasmacytosis to globulin and antibody production is substantiated by the experimental literature. Downey's (1911) description of a plasmacytosis in the bone marrow of a rabbit with an incidental nonspecific subcutaneous abscess initiates the series. Doan, Sabin, and Forkner (1930) and Miller (1931) produced omental plasmacytosis by repeated intraperitoneal administration of tuberculo proteins. Rich (1935 and 1936) produced acute splenic tumor in rabbits by horse serum. The characteristic feature was the "acute splenic tumor cell." In similar preparations made in this laboratory we have identified these cells as developing plasma cells. Kolouch (1938)

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studied the plasma cell and found it in the bone marrow of ten species of laboratory animals and also showed that the plasma cell content of the rabbit bone marrow could be experimentally increased by sensitization and shock with *Streptococcus viridans*. He demonstrated the development of the bone marrow plasma cell from the reticular plasma cell in a period of five days and postulated a relation between this transformation and the development of antibodies. Björneboe and Gormsen (1941) described the experimental production of a hyperglobulinemia in rabbits by intensive immunization with different types of formalin-killed pneumococci. They found a concomitant increase in plasma cells in various tissues. They postulated that the increase in the globulin fraction of the blood was due to the increase in antibodies and that this in turn was related to plasmacytosis.

The purpose of the following experiments is to elucidate the relationship of the bone marrow plasma cells to the reticulo-endothelial cell and to the phenomena of sensitivity and shock. Both bacterial and simple protein antigens have been used. Illustrative material from a case of serum sickness is included.

MATERIALS AND METHODS

Rabbits were used in all experiments. Series 1* consists of six rabbits which were injected repeatedly over a period of several months with varying doses of *Str. viridans*. Series 2 contains three rabbits injected subcutaneously with a single dose of 10 c.c. of a culture of *Str. viridans* suspended in agar. After a period of five weeks they were shocked with broth culture of the same organism and serial rib biopsies were made at intervals up to five days. Series 3 consists of sixteen animals which were sensitized to egg white by its injection on alternate days in the following dosages: 1 c.c., intravenously; 0.5 c.c., intravenously; and 1 c.c., intramuscularly. Four animals were killed following the sensitization and four immediately after shock, which was induced twenty to thirty days later by injection of 0.4 c.c. egg white intravenously; four were killed on the fourth day following shock by injection 0.4 c.c. egg white; while the bone marrow of the remaining four was sampled before sensitization, after sensitization, following anaphylactic shock, and periodically thereafter for four days.

The bone marrow was imprinted and stained with Wright-Giemsa.

EXPERIMENTAL RESULTS

The six rabbits of series 1 which were treated over a period of months with multiple injections of *Str. viridans* all showed marked increase in the bone marrow plasma cells, and in some animals there was noted an increase in the reticular plasma cells. These changes were critically followed in the three animals of the second series. Rib bone marrow biopsies were made before sensitization and the elements compared with similar material from the same subjects after sensitization and following shock. The control bone marrows show few reticular plasma cells. These are found to be increased in the biopsies taken after sensitization. They appear as large cells with basophilic, rather muddy cytoplasm with eccentric nuclei characterized by a nuclear pattern similar to that seen in reticular cells (Fig. 2,*a*).

In these preparations the chromatin appears as a fine purplish-violet dust sprinkled on a background of pinkish-yellow karyoplasm (parachromatin). The chromatin granules are uniform, finely divided, round purplish-violet particles.

*The material for this series was obtained from Dr. B. J. Clawson.

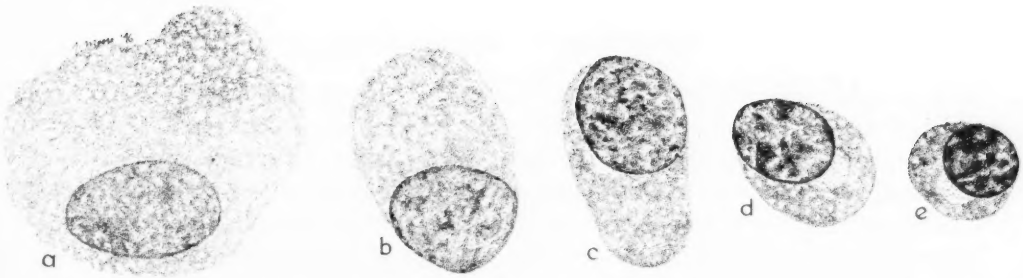

Fig. 1.—Representative plasma cells imprinted at biopsy from bone marrow of rabbit sensitized to egg white. *a*, Reticular plasma cell before anaphylactic shock. *b*, Initial transformation stage four hours after shock. *c*, Additional shrinkage and metamorphosis after eight hours. *d*, Medium-sized plasma cell at forty-eight hours. *e*, Typical Marshalko type plasma cell after ninety-six hours.

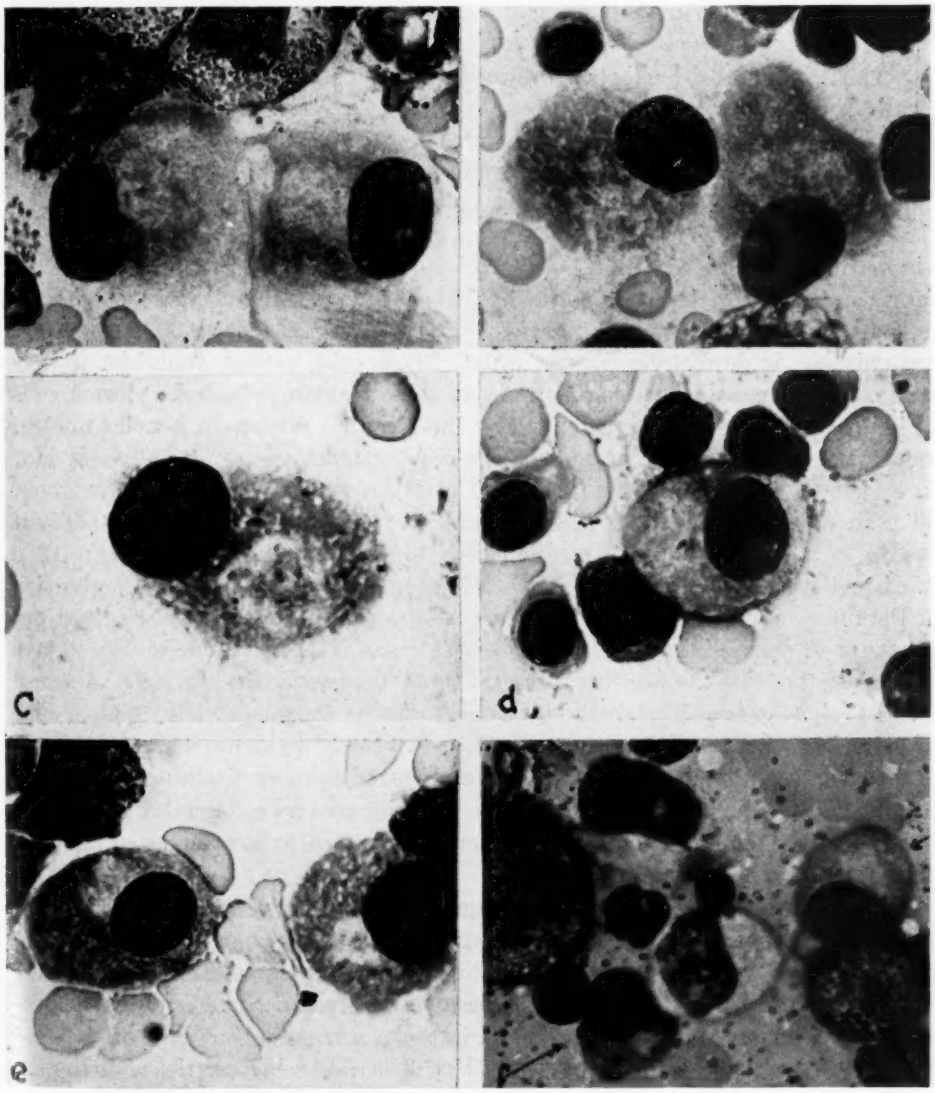

Fig. 2.—Photomicrographs of cells showing stages in metamorphosis from reticular plasma cells to Marshalko type plasma cells. Rabbits sensitized and shocked with *Str. viridans*. *a* and *b*, Group of plasmacellular reticulum cells before shock. *c* to *f*, Transformation stages to Marshalko type plasma cells.

Each one is surrounded by and sharply demarcated from the parachromatin which it tends to mask. The chromatin pattern is more condensed and the nuclear membrane slightly thicker than in the unaltered reticulum cell. In the cytoplasm the basophilic spongioplasm occurs as minute masses which are arranged to form small coalescing bars. These elongated clumps are surrounded by and sharply separated from an abundant yellowish hyaloplasm. The arrangement of these two components of the cytoplasm gives it its characteristic canaliculated appearance. In the region of the nucleus there is frequently seen a lighter staining area (increase of hyaloplasm) which suggests a Hof. When occurring alone the cells appear irregularly elliptical, but most frequently they are seen in clumps of five to fifteen cells, the cytoplasmic boundaries then being rather indistinct.

The initial transitional stage occurs within two hours after shock (Fig. 2, *b*). The boundary of the cytoplasm becomes more clearly defined and rounding of the cells is initiated. In the nucleus contraction occurs and a coarser chromatin pattern is to be observed. At four hours shrinkage of the cytoplasm is noted and the basophilia is more apparent than before. By this time dispersion of the cells which previously had been clumped has taken place, and the reticular plasma cells are mixed with the other bone marrow elements. Shortly after four hours the Hof becomes marked. This together with the more condensed nucleus and increased basophilia of the spongioplasm leads to cells more easily recognizable as developing plasma cells. These cells become abundant at five hours. In the ensuing five hours, many of these transform to large plasma cells (Fig. 2, *c*). These are characterized by the eccentric nucleus, a heavier nuclear membrane, blocklike arrangement of the chromatin masses, and a distinct Hof in a still more basophilic cytoplasm. These large plasma cells measure about 20 μ in diameter. Their further evolution consists of shrinkage in the next twelve to thirty-six hours to medium-sized plasma cells measuring about 12 μ in diameter (Fig. 2, *d* and *e*). With this decrease in size, there is accentuation of the nuclear pyknosis, the cytoplasmic basophilia, the Hof, and the initial appearance of the radkern nuclear pattern. These cells are abundant two days following the shock. Continuation of these tendencies results in the small plasma cell (the true Marschalko type) abundant at four days (Fig. 2, *f*). Some of these cells become, after the fifth day, extremely pyknotic and apparently degenerate, their nuclear structure being obscured by their staining properties.

At no time during this cycle does the reticular plasma cell exhibit phagocytic properties, though other cells of the reticulum are seen to have ingested bacteria and erythrocytes.

To serve as control for the bacteremia and local infection of the first two series, as well as to test the general nature of the phenomenon, the sixteen rabbits of series 3 were sensitized to egg white and studied before and after shock. The observations differ little from those obtained using *Str. viridans* as antigen. In the four animals killed before shock, a massive increase of plasma-cellular reticulum cells may be seen (Fig. 3, *a*). As before, the cells appear in clumps and show morphology identical to those previously described. In the four animals that died immediately upon shock, no change from the pre-

shock picture is noted. Those which were killed four days after shock show marked plasmacytosis of the bone marrow (Fig. 3, *b*). Serial biopsies of the remaining four at 2,4,8,14,24,48,72, and 96 hours, as well as pre- and post-sensitization controls, confirm in all respects the transformation from the reticular plasma cell to the plasma cell previously described (Fig. 1).

That this same transformation from reticular plasma cell to plasma cell occurs in human beings is shown in Fig. 4, which is a blood smear taken from a boy suffering from serum sickness resulting from the administration of tetanus antitoxin.

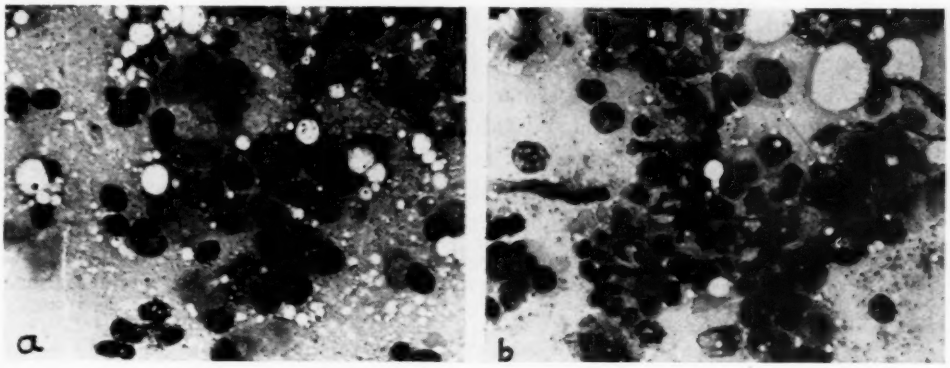

Fig. 3.—Bone marrow imprints from rabbit sensitized to egg white. *a*, Clumped reticular plasma cells before shock. *b*, Marshalko type plasma cells four days after shock.

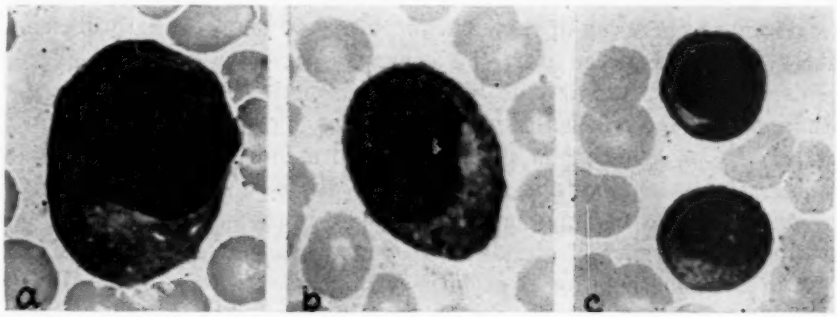

Fig. 4.—Illustration of intermediate stages between reticular cells and plasma cells as seen in blood smear of patient with serum sickness. *a*, Cell with reticular type nucleus. *b*, Condensation of nucleus. *c*, Further condensation of nucleus and cytoplasm to close approximation of plasma cell.

DISCUSSION

These experiments show two phenomena of interest. There is an increase in a specific type of bone marrow reticulum cell following sensitization to an antigen. Second, anaphylactic shock initiates a morphologic cycle by which the reticular plasma cells evolve to plasma cells. We have noted the development of similarly striking plasmacytosis in the other reticulo-endothelial organs following sensitization and shock with bacterial and other antigens. In view of the evidence in the literature and our own experiments, it is highly suggestive that the transformation of the plasmacytic reticulum cell to the Marshalko type

plasma cell is intimately associated with the production of antibody protein. It is consistent with recent findings (Dougherty and White, 1946, Dempsey and Wislocki, 1946) to presume that the basophilia of the early plasmacyte may be associated with heightened protein formation. In view of the needs of the organism at this time, antibody production might well be carried on in such active cells. Certain it is that the loss of volume is a most striking cellular change in the procession of stages from basophilic reticulum to small plasma cell. Yet, the impression is gained that stainable material in these preparations is not reduced. A loss of the fluid components of the cell is obvious. It would be hard to overlook, however, the possible relation of the antigen-antibody reaction to the permeability of the cell membrane in searching for a possible mechanism for these changes. If serum hyperglobulinemia is directly related to plasma cell development, it is likely that some of the cell shrinkage may be due directly to loss of protein elements in the form of globulin. Morphology reaches its limitations in this type of work and must be supplemented by other methods. Before a thorough understanding of this matter is to be had, we must know whether, in tissue culture, the cell changes under question are associated with the production of antibodies. In addition, the role of the various cell organelles in the reaction to anaphylactic shock must be worked out on a cytochemical basis.

Another aspect of the problem which must remain speculative until more detailed information is available is the nature of the stimulus furnished by anaphylactic shock, which brings about the sequence of cell transformations that produces Marschalko plasma cells from a much less differentiated type of cell. The spectacular operation of this cytomorphosis not only serves to illustrate the exquisite relation between physiologic changes and the ontogeny of cellular elements, but it also promises to serve as an experimental preparation for the determination of the relation of nuclear changes to cytoplasmic activity.

Markoff's work on serum sickness, as well as our case, serves to illustrate the clinical importance of the matter under discussion. It is apparently the exceptional case in which plasma cells are found in the peripheral blood, but the concomitant appearance of circulating reticular plasma cells indicates that the process in the bone marrow is essentially the same as in the bone marrows of experimental animals. It should be noted that in the rabbits, circulating plasma cells were not regularly found, and we may believe that some secondary factor in our clinical case brought the cells into the peripheral blood.

SUMMARY

1. An analysis of the literature provides ample evidence to link plasmacytosis to antibody production.

2. With *Str. viridans* and egg white as antigens, sensitization of rabbits was found to increase enormously the number of reticular plasma cells in the bone marrow.

3. Shocking the animals with the antigen initiated a cycle of nuclear and cytoplasmic condensation, visible in two hours and extending to four days, by which the reticular plasma cells are transformed to Marshalko type plasma cells.

4. In a case of human serum sickness, both reticular plasma cells and various stages of the plasma cell appeared in the peripheral blood.

5. It is suggested that this morphologic cycle is associated intimately with the production of antibodies.

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HEMATOPOIESIS IN THE BONE MARROW OF RATS RECOVERING FROM NUTRITIONAL ANEMIA

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INTRODUCTION

THE present paper is a report on the results of studies of the hematopoietic activities found in the bone marrow of rats made anemic by being fed a diet composed of milk only and then caused to recover from the anemia by being fed copper and iron as supplements to the milk diet. Studies of the recovery of rats made anemic by this method have seldom included observations on changes in the number of leucocytes but have chiefly been concerned with the regeneration of hemoglobin and erythrocytes and with the agents which induce regeneration of those blood elements. The level of hemoglobin and the number of erythrocytes of the circulating blood have been employed as indicators of the severity of the anemia produced by feeding the milk diet, and the rate and degree of regeneration of those hematologic elements have been used to measure the curative properties of substances added to the milk diet. Through the work of Hart, Steenbock, and Elvehjem and co-workers,[1-9] Beard and Myers and associates,[10-17] Mitchell and Miller,[18] Hamre and Miller,[19, 20] Smith and Otis,[21] Stein and Lewis and co-workers,[22-25] and others, it has been established that copper and iron when fed simultaneously in adequate quantities as supplements to the milk diet will cause recovery from the anemia.

Studies of the blood-forming organs of rats in nutritional anemia and of rats recovering from the anemia have been few in number. Von Haam and Beard[26] found the spleen of rats made anemic by being fed a diet of milk only to be small, pale, and soft. The spleens of the anemic animals possessed fewer and smaller follicles, a decreased perifollicular zone, and pulpar cells which showed signs of degeneration. Hamre and Miller[19] also found the spleens of anemic rats to be small in size and to possess a decreased amount of white pulp tissue, the decrease in the extent of the tissue being due to a loss of the outer zone and part of the inner zone of the Malpighian corpuscles and of the outer portions of the periarterial white pulp. The red pulp was increased in area and included circulating mature red blood cells and normal reticular cells. There was no indication of cell multiplication in the spleens of the anemic animals. The latter authors also demonstrated that, during the recovery from the anemia following iron and copper therapy, the spleens of the animals became temporarily enlarged and mottled with grayish-white hematopoietic areas. When approximately normal values of the blood were restored, the spleens of the recovering animals decreased to normal size. The white pulp

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Published with the permission of the Director of the Hawaii Agricultural Experiment Station as Technical Paper No. 152.

tissues were regenerated and restored to normal histologic appearance during the period of enlargement, and the normal appearance of the white pulp was retained when the spleens returned to normal size. Active erythropoietic tissue occupied the red pulp areas of the enlarged spleens, and in the early periods of recovery those areas included large numbers of hemocytoblasts while in the later periods of recovery the areas were composed largely of polychromatophilic normoblasts. The erythropoietic areas decreased in extent and disappeared in the later periods of recovery. In a later study Hamre and Miller[20] demonstrated that removal of the spleens of anemic animals did not prevent the recovery of hemoglobin and erythrocytes under copper and iron therapy. However, there were indications that the rate of recovery of the erythrocytes was slower for the splenectomized than for the nonsplenectomized animals.

The paper by Fitz-Hugh and associates,[27] as far as the present writer has been able to determine, is the only paper in the literature on the nutritional anemia of the rat which presents observations on some of the characteristics of the bone marrow of anemic animals and of animals recovering from the anemia. These authors made observations on the quantity of fatty and cellular tissue of the marrow and pointed out that the cellularity of the marrow of normal animals was greater than that of anemic animals. They also noted that the addition of curative agents to the milk diet caused an increase in the cellularity of the marrow of all of their treated animals. For animals fed iron alone as supplement to the milk diet, the hyperplasia of the marrow tissue persisted throughout a seventy-day period of therapy. The marrow of anemic animals fed iron and copper, or iron and sodium glutamate, became highly hyperplastic in the early stages of recovery but in the later stages became less cellular than the marrow of normal animals. Though the paper[27] did indicate that gross microscopic changes of the bone marrow of anemic animals did follow therapy, it did not describe the cytology of the marrow tissues or point out the nature of the hematopoietic response to therapy.

Because of the marked hematopoietic response of the spleen and the rapid restoration of normal blood values which followed the feeding of therapeutic agents, the present writer assumed that the bone marrow of anemic animals similarly would exhibit a sharp hematopoietic response to therapy. Preliminary studies of the bone marrow of animals killed at daily intervals during recovery from anemia were carried out, and, though an increase in the cellularity of the marrow was found, the cytologic characters noted indicated that only hematopoiesis of the homoplastic type occurred. However, the observations of Hamre and Miller[20] that marked increases of normoblasts and leucocytes of the circulating blood occurred on the second and the fifth or sixth day of therapy suggested that a marked change of hematopoiesis of the bone marrow might occur during the first day of feeding of the iron and copper supplements. Study of the bone marrow of rats killed at hourly intervals during the first two days of supplement feeding did show a marked hematopoietic response to the therapy. The present paper describes the hematologic changes found to occur in the bone marrow and the blood of anemic animals during the first forty-eight hours of the recovery period.

MATERIALS AND METHODS

Sixty animals divided into three groups of twenty each were observed in these studies. Though the three groups of animals were placed on experiment at different times, to establish identical conditions for study, all animals were managed in the same manner. All animals were made anemic by the method outlined by Elvehjem and Kemmerer,[28] that is, at the age of 21 days they were weaned and placed on a diet of cow's milk. The milk was obtained at the University Farm and was collected directly into glass bottles and fed to the animals in glass dishes. The animals were kept in individual galvanized iron wire cages. When the hemoglobin value had decreased to 3.5 Gm. or less, per 100 c.c. blood, the animals were given iron and copper as supplements to the milk. Eighteen of the animals of the first group were fed the iron and copper supplements after 28 to 32 days on the milk diet while the remaining two animals of the group were kept on the milk diet for 44 days. The animals of the second and third groups, though they possessed satisfactorily low hemoglobin values after 28 days on the milk diet, were continued on the deficient diet for 36 to 54 days in the hope that the hemoglobin and erythrocyte values would be further reduced and a more severe anemia established. Though the animals continued on the milk diet for the longer periods did become progressively weaker, only slight decrease of hemoglobin and erythrocyte values occurred for some but not all animals. Each animal fed the copper and iron supplements received a single dose of the curative agents unless they were continued under observation for more than 24 hours when they were given a second dose of the supplements at the beginning of the second 24-hour period. A single dose of the curative agents consisted of 0.5 mg. iron for all individuals and copper fed at the rate of 0.003 mg. per gram body weight. The iron was supplied as ferric chloride ($FeCl_3.6H_2O$) and the copper as cupric sulfate ($CuSO_4.5H_2O$). The copper and iron content of the various solutions used were checked by the colorimetric method.

The first group of twenty animals was employed as a part of a preliminary experiment to determine approximately at what time during the early stages of recovery critical or significant phases of hematopoiesis might occur. Because of the erythropoietic function of the spleen during recovery, it was decided that anemic animals with large, actively erythropoietic spleens should not be used in this study. Each animal, therefore, was subjected to laparotomy and the size and appearance of the spleen were noted. All animals were used in the experiment because the spleens were small or medium sized and none possessed grayish-white erythropoietic areas. Two of the animals, to serve as controls, were killed without having received copper and iron supplements. Seventeen of the animals were fed iron and copper and one of these was killed at each of the following hours after copper and iron feeding: 2, 3, 5, 7, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 36, and 48 hours. The twentieth animal of this group was bled hourly from the fourteenth to the twenty-eighth hour after feeding to provide hourly blood values.

Since none of the animals of the first group possessed large and actively erythropoietic spleens and laparotomy introduced a complicating factor into the experiments, none of the animals of the second and third groups were subjected to exploratory operation. Five of the animals of these groups were killed as anemic control animals. Since the animal of the first group killed at 22 hours after iron and copper feeding possessed a bone marrow showing a particularly sharp response to therapy, the animals of the second and third groups and which received iron and copper, were killed at some hour between 10 and 48 hours after supplement feeding.

Hemoglobin, erythrocyte, leucocyte, and platelet values were determined for all control animals and for all treated animals at the time they were given their first dose of copper and iron and again at the time they were killed. All blood samples were obtained from the rat's tail by the usual methods. Hemoglobin values were determined by the acid hematin method employing a Klett-Summerson photoelectric colorimeter. The erythrocyte and leucocyte counts were made by the usual methods and the platelet counts by the method of Reese and Ecker. At the time blood samples were taken for blood values, blood smears were also prepared. The blood smears were stained with Wright's stain and Giemsa stain. At the time the animals were killed, the femur, humerus, vertebrae of the base of the tail,

and vertebrae from the middle of the free portion of the tail were cleaned of muscles and connective tissue and fixed in Helly's Zenker-formol fixative. The marrow tissue of one femur was removed and a portion used to prepare smears and imprints and the remainder fixed for sectioning. The smears of the bone marrow were stained with Wright's stain followed by Giemsa stain. Entire bones and the marrow removed from the femur fixed for histologic sections were embedded in celloidin, decalcified, sectioned, and stained in hematoxylin eosin, hematoxylin-eosin-azure II, or Dominici's toluidine blue-eosin-orange G stain.

OBSERVATIONS AND RESULTS

The results of this investigation will be presented in two parts, the first dealing with the values for the hemoglobin and blood cells of anemic animals and animals given iron and copper supplements and the second with the histology of the bone marrow of anemic and treated animals. Observations on the values of the blood elements are presented for the purpose of indicating the characteristics of the anemia investigated in this study and because the changes of the values of the blood elements which follow treatment with copper and iron help explain the histologic changes found in the bone marrow. The relation of the hematologic conditions observed to the controversial problems and theories of hematopoiesis will be presented in the discussion which follows this section of the paper.

The anemic animals were physically weak and inactive and many of the animals, often in spite of high platelet counts, showed a tendency toward failure of blood coagulation, the bleeding from the tail after taking blood samples being difficult to control. The average values and range of values of the blood elements of the anemic animals are summarized in Table I. The range of values recorded for hemoglobin and erythrocytes and the presence of normoblasts in the blood smears indicate that the anemia for all animals was a severe one. Though the total number of leucocytes was reduced below normal, the absolute values given for the various types of leucocytes show that the differential leucocyte count was approximately normal and the lymphocyte the predominant leucocyte type of the blood smear. The platelets, as shown by the range of values listed in Table I, were extremely variable in number for our anemic animals.

The anemia produced by feeding milk to the rats of this experiment was of the microcytic, hypochromic type. Anisocytosis was marked and the blood smears showed quite clearly that the greater number of erythrocytes was smaller than normal. An average of 4.5 μ was obtained by employing a diffraction erythrocytometer for measuring the diameter of the erythrocytes of the blood smears of thirty-six of the anemic animals. Though there is no agreement in the published values for the diameter of the red cells of normal rats, the values of 5.7 μ given by Kleineberger,[29] 6.3 μ given by Scarborough,[30] and 6.8 μ given by Gulliver and quoted by Ponder[31] are all greater than the average diameter obtained for our animals. The erythrocytes of the blood smears were either strongly basophilic, polychromatophilic, or possessed a faintly stained narrow acidophilic margin. Basophilic stippling of the red cells was present and Jolly bodies were encountered quite frequently in the blood smears. Poikilocytosis was either absent or slight.

TABLE I. SUMMARY OF BLOOD VALUES OF ANEMIC RATS AND RATS AFTER COPPER AND IRON THERAPY

BLOOD ELEMENT	AVERAGE VALUES*		RANGE OF VALUES*	
	ANEMIC ANIMALS ON DAY KILLED AS CONTROLS OR FED COPPER AND IRON SUPPLEMENT	TREATED ANIMALS KILLED FROM 2 TO 48 HOURS AFTER FIRST FEEDING OF COPPER AND IRON	ANEMIC ANIMALS ON DAY KILLED AS CONTROLS OR FED COPPER AND IRON SUPPLEMENT	TREATED ANIMALS KILLED FROM 2 TO 48 HOURS AFTER FIRST FEEDING OF COPPER AND IRON
Hemoglobin in grams per 100 c.c blood	2.8 (54†)	3.3 (46)‡	Less than 2.0 to 3.8 (59)	Less than 2.0 to 5.2 (50)
Erythrocytes in millions per c.mm. blood	2.05 (59)	2.22 (52)	1.16 to 2.92 (59)	1.29 to 3.41 (52)
Leucocytes in thousands per c.mm. blood	5.37 (57)	16.44 (50)	2.71 to 11.65 (57)	3.16 to 42.71 (50)
Platelets in thousands per c.mm. blood	409 (40)	433 (36)	96 to 814 (40)	80 to 926 (36)
Normoblasts in number per c.mm. blood	208 (57)	2121 (50)	0 to 2063 (57)	0 to 8426 (50)
Heterophile in number per c.mm. blood	2060 (57)	10,171 (50)	444 to 4943 (57)	1408 to 32,708 (50)
Eosinophiles in number per c.mm. blood	78 (57)	429 (50)	0 to 331 (57)	0 to 2431 (50)
Lymphocytes in number per c.mm. blood	3433 (57)	5359 (50)	1005 to 6156 (57)	1256 to 17,738 (50)

*Numbers in parentheses indicate the number of animals observed for each blood element.

†Five additional animals had less than 2.0 Gm. hemoglobin per 100 c.c. blood and, therefore, below the lowest calibration of the colorimeter. These animals were not included in the computation of the average value.

‡Four additional animals had less than 2.0 Gm. hemoglobin per 100 c.c. blood and, therefore, below the lowest calibration of the colorimeter. These animals were not included in the computation of the average value.

In this study the anemic animals given recovery treatment were killed a few hours after the initial copper and iron feeding and, therefore, did not show marked improvement in general physical condition. However, the animals continued to the end of the 48-hour observation period were more active than before receiving copper and iron. The anisocytosis did not disappear and in some instances was more marked after therapy than before. Likewise, the microcytosis was continued, the average diameter of the erythrocytes of thirty-six treated animals being 4.5 μ. The basophilic erythrocytes in some instances were increased in number and in general the hypochromasia continued unchanged after therapy. From these observations it is apparent that for the period of the first 48 hours after the initial copper and iron feeding, the hypochromic and microcytic character of the anemia was continued and was not markedly altered.

The values for the hemoglobin and blood cells of the animals fed copper and iron are summarized in Table I. Though the average values and range of values presented in the table are for all animals fed the curative substances and does not take into consideration the fact that the animals were killed at various times after supplement feeding, they do indicate the character of the response to therapy. Slight increases in value are indicated for hemoglobin, erythrocytes, and platelets, while marked increases in number are shown for normoblasts and leucocytes. The great increase in number of white blood cells is shown to be due primarily to an increase in number of heterophile leucocytes.

Though lymphocytes were increased over their values for untreated anemic animals, their increase in number was small when compared to the increase in number of heterophile leucocytes. As a result the differential leucocyte count was altered, the heterophile becoming the predominant leucocyte type after treatment. The marked increase in number of normoblasts, eosinophiles, and heterophiles shows that the first response to copper and iron feeding is myeloid in nature and characterized by a discharge of those cells into the circulating blood by the bone marrow.

Examination of the hemoglobin and blood cell values for animals killed at different hours after the initial feeding of copper and iron shows that a latent period of no regular gain immediately follows the feeding. It also shows that the latent period continues to 26 hours for hemoglobin and 28 hours for erythrocytes, while for normoblasts and leucocytes it continues only to 12 hours after feeding. The duration of the latent period is shown particularly well by the gain or loss in values shown in the graphs of Figs. 1 and 2. Since the platelets did not show a definite regular response to therapy, their changes in values were not graphed and included in the illustrations.

Though Krumbhaar,[32] Wintrobe,[33] and others state that due to an increase in number of neutrophiles, a postoperative leucocytosis is found to occur, the values for leucocytes of our animals subjected to laparotomy do not differ significantly from the values of animals not subjected to operation. We have concluded that the change in numbers of normoblasts and leucocytes of the circulating blood of our animals was due to the copper and iron feeding. We have also concluded that the increase in number of those cells was due to their discharge by the bone marrow and that they were discharged during the period of high and low values extending through the period between 12 to 26 hours after copper and iron feeding. That period we regard to be a critical period of response to therapy and, as will be described, is accompanied by critical changes of hematopoiesis in the bone marrow.

The bones whose marrow tissues were observed for histologic changes in this investigation, on the basis of position in the body, were of two types, outlying or distal bones represented by the caudal vertebrae and central bones represented by the femur and the humerus. Huggins and Blocksom[34] have pointed out that in the rat the marrow of the outlying bones is characteristically of the hematopoietically inactive yellow type while that of the central bones is of the hematopoietically active red type. We assumed that by including representative bones of the two types we would be presented the opportunity of noting the reaction of both yellow and red marrow to anemia and to therapy. The marrow tissues of the two types of bones were found to be dissimilar both in the anemic state and after therapy and, therefore, require separate consideration and description.

Longitudinal sections of vertebrae from two portions of the tail, namely, vertebrae of the middle of the free portion of the tail and vertebrae immediately following the sacral vertebrae at the base of the tail, were prepared for histologic study of the marrow. The marrow tissue of the vertebrae of the two regions of the tail presented some points of difference. The marrow of the

distal caudal vertebrae, except for a few islands of hematopoietic tissue in the epiphyses and metaphyses, was of the yellow variety throughout. The marrow of these vertebrae resembled that described by Huggins and Blocksom[34] for normal adult rats, and it is apparent that in our animals the existence of a severe anemia had not produced active hematopoietic tissue in these vertebrae. The marrow of the distal caudal vertebrae showed no histologic changes in response to copper and iron therapy. The first caudal vertebra of anemic

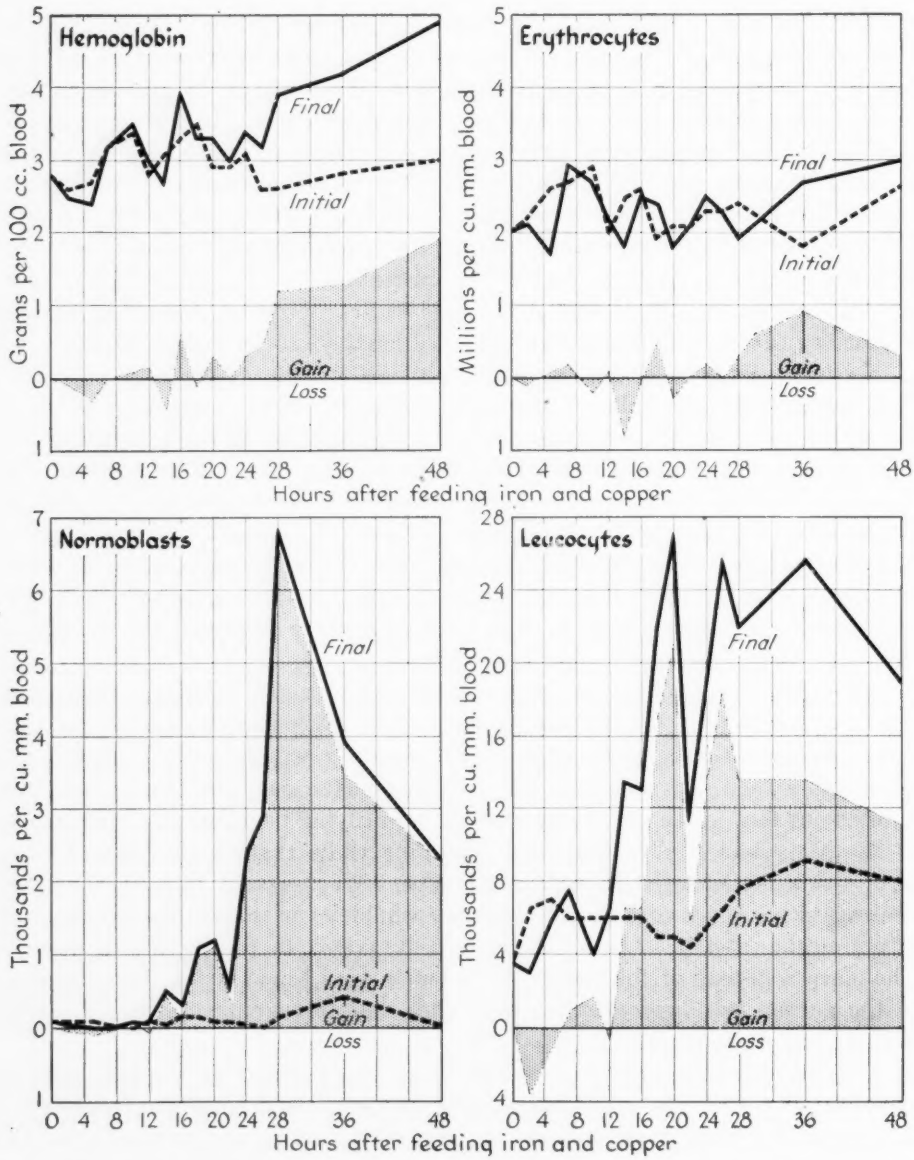

Fig. 1.—Effect on hemoglobin erythrocytes, normoblasts, and leucocytes of feeding a total of 0.5 mg. iron and 0.003 mg. copper per gram body weight.

animals possessed fairly abundant red marrow in both epiphysis and centrum. Fat cells were present in the marrow and were most abundant toward the posterior end of the vertebra. The first caudal vertebra possessed more red marrow and less yellow marrow than the second and third caudal vertebrae, and the third vertebra possessed less red and more yellow marrow than the second vertebra. The third caudal vertebra, therefore, possessed more yellow marrow than red marrow. It should be noted that for the second and third

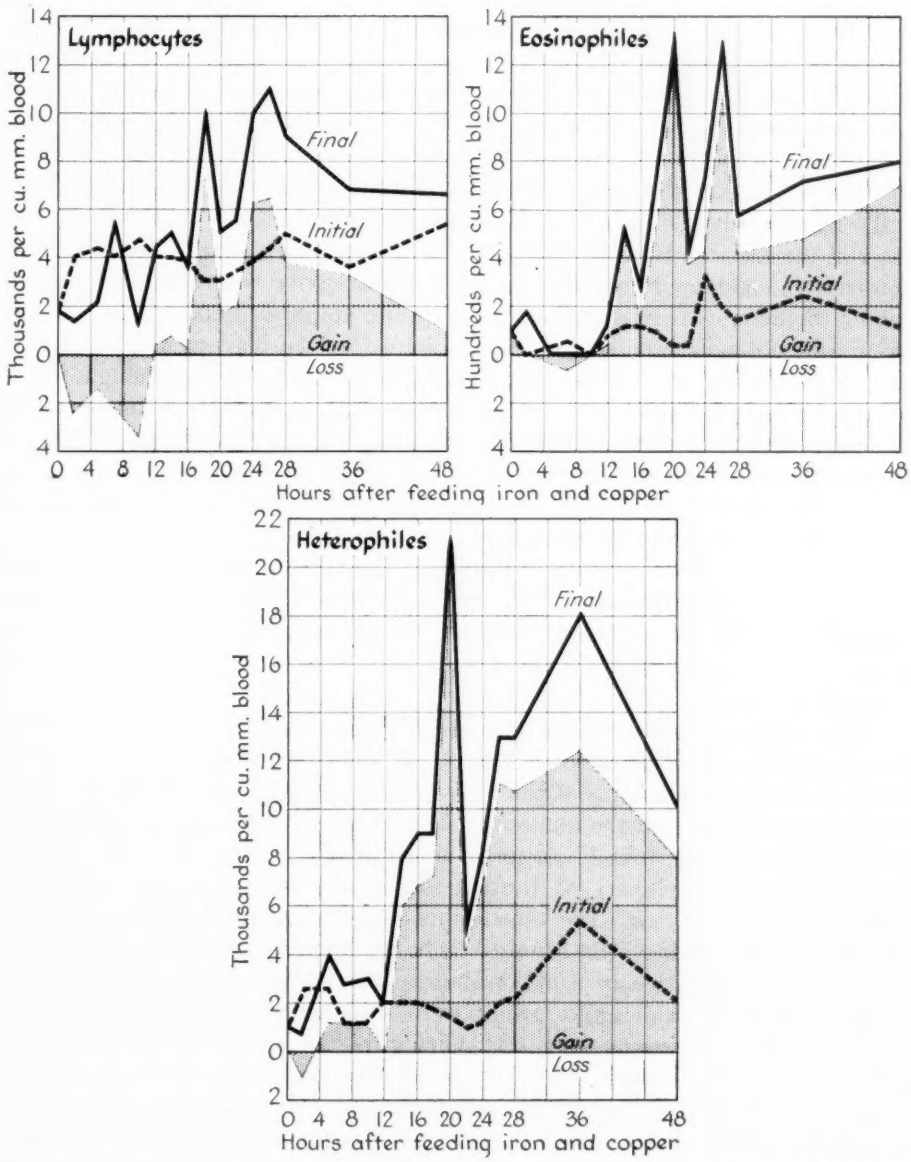

Fig. 2.—Effect on lymphocytes, eosinophiles, and heterophiles of feeding a total of 0.5 mg. iron and 0.003 mg. copper per gram body weight.

caudal vertebrae the red marrow was best developed and most active at the anterior end. The decrease in red marrow and increase in yellow marrow from the first to the third caudal vertebrae were anticipated because the first vertebra is essentially a central type of bone while the third caudal vertebra is essentially an outlying type of bone. Though histologic changes were found to occur in the red marrow of the first and to some extent in the second caudal vertebrae after therapy, the changes were not as extensive or as marked, though of the same nature, as those found to occur in the femur and humerus. For that reason further description of the marrow of the caudal vertebrae will not be given. Our observations indicate that the severe dietary anemia of our study did not cause the extension of red marrow into typical yellow marrow areas and that typical yellow marrow does not, for the 48-hour period of observation of this study, respond to copper and iron therapy.

The sections of the marrow removed from the femur before being placed in the fixative when stained with hematoxylin-eosin-azure II provided the best material for the identification and study of the various stages of hematopoiesis. However, the marrow removed from the marrow cavity before fixation did not include the marrow tissues at the periphery and epiphyseal ends of the marrow cavity and did not provide a complete picture of the marrow tissue. Longitudinal sections of the entire femur and also of the humerus, though not as sharply stained with the hematoxylin-eosin-azure II, provided excellent material for study of the cytology of red marrow as well as for the extent of the red marrow tissues. The marrow tissue of the femur and humerus, both in the anemic state and after therapy, were essentially similar as far as hematopoietic activity was concerned and were used to supplement each other in this study. The descriptions of the cytologic changes of the marrow to be given are based largely on the longitudinal sections of the entire femur and of the marrow removed from the femur before fixation. The smears and imprints were very useful in identifying hematopoietic cells and were used to supplement the sectioned material.

The histology and cytology of typical femur marrow of anemic rats of this experiment are illustrated in Fig. 3. It should be noted that the marrow tissue of anemic animals was of the hyperplastic red type throughout the entire extent of the femur and the humerus. Fat cells were very few in number and were most commonly found, scattered singly or in groups of a few cells, in the tissue at the periphery of the marrow of the central cavity and in the marrow of the epiphyses. The venous sinuses were prominent and well defined and the reticular lining cells forming their walls were continuous and distinct. There was no indication of a discontinuous wall of the venous sinuses in any of our sections in which the tissues were satisfactorily stained. The intersinusoidal tissue, or parenchyma, was sharply limited by the reticulo-endothelial walls of the venous sinuses and toward the central portion of the tissue were found the arteries and arterioles supplying it. The parenchymatous tissue was fairly cellular and compact though open enough to permit easy identification and study of the reticular cells forming its framework. Some of the reticular cells were in the process of mitosis and others, as shown by the lack

Figs. 3, 4, and 5.

Sections of marrow of femur of untreated anemic rat (Fig. 3), anemic rat killed 22 hours after receiving 0.5 mg. iron and 0.22 mg. copper (Fig. 4), and anemic rat killed 48 hours after receiving 0.5 mg. iron and 0.26 mg. copper (Fig. 5).

All tissues fixed in Zenker-formol and stained in hematoxylin-eosin-azure. Camera lucida drawings, ×1100.

In all cases the abbreviations have the following meaning: *B.*, Basophile; *P.*, polychromatophile; *Pl.*, plasma; *O.*, orthochromatophile; *Eos.*, eosinophile; *Eryth.*, erythrocyte; *Hemocyt.*, hemocytoblast; *Het.*, heterophile; *Megak.*, megakaryocyte; *myel.*, myelocyte; *metamyel.*, metamyelocyte; *norm.*, normoblast; *Plt.*, platelet; *Pronorm.*, pronormoblast; *Ret.*, reticular; *V.*, venous.

Eosinophile (*Eos. 1*, Fig. 4) and polychromatophile normoblast (*P. norm. 1*, Fig. 4) entering venous sinus; pronormoblast in mitosis (*Pronorm. 1*, Fig. 4); megakaryocytes in mitosis (*Megak. 3*, Fig. 5), showing platelet formation (*Megak. 2*, Fig. 5), and in degeneration (*Megak. 1*, Fig. 3); group of normoblasts in homoplastic development (*Myelopoietic group 1*, Fig. 5) and heterophiles in homoplastic formation (*Myelopoietic group 2*, Fig. 5); reticular cells lining venous sinuses (*Ret. cell 1*, Figs. 3, 4, and 5), reticular cells of stroma (*Ret. cell 2*, Figs. 3 and 4), stroma reticular cell in mitosis (*Ret. cell 3*, Fig. 3), reticular cell transforming into hemocytoblast (*Ret. cell 4*, Fig. 3), and stroma reticular cell bordering on lining cell of venous sinus (*Ret. cell 5*, Fig. 5).

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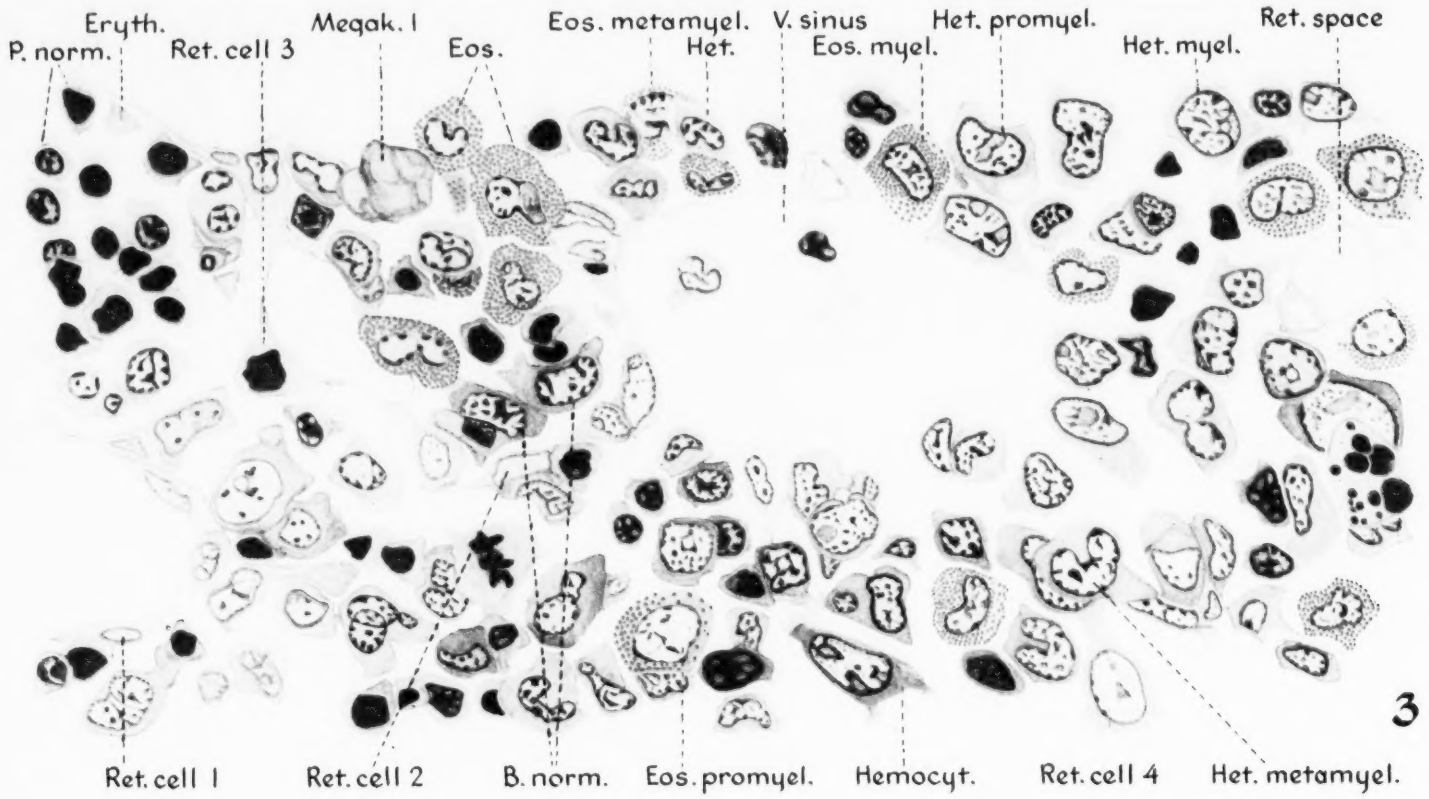
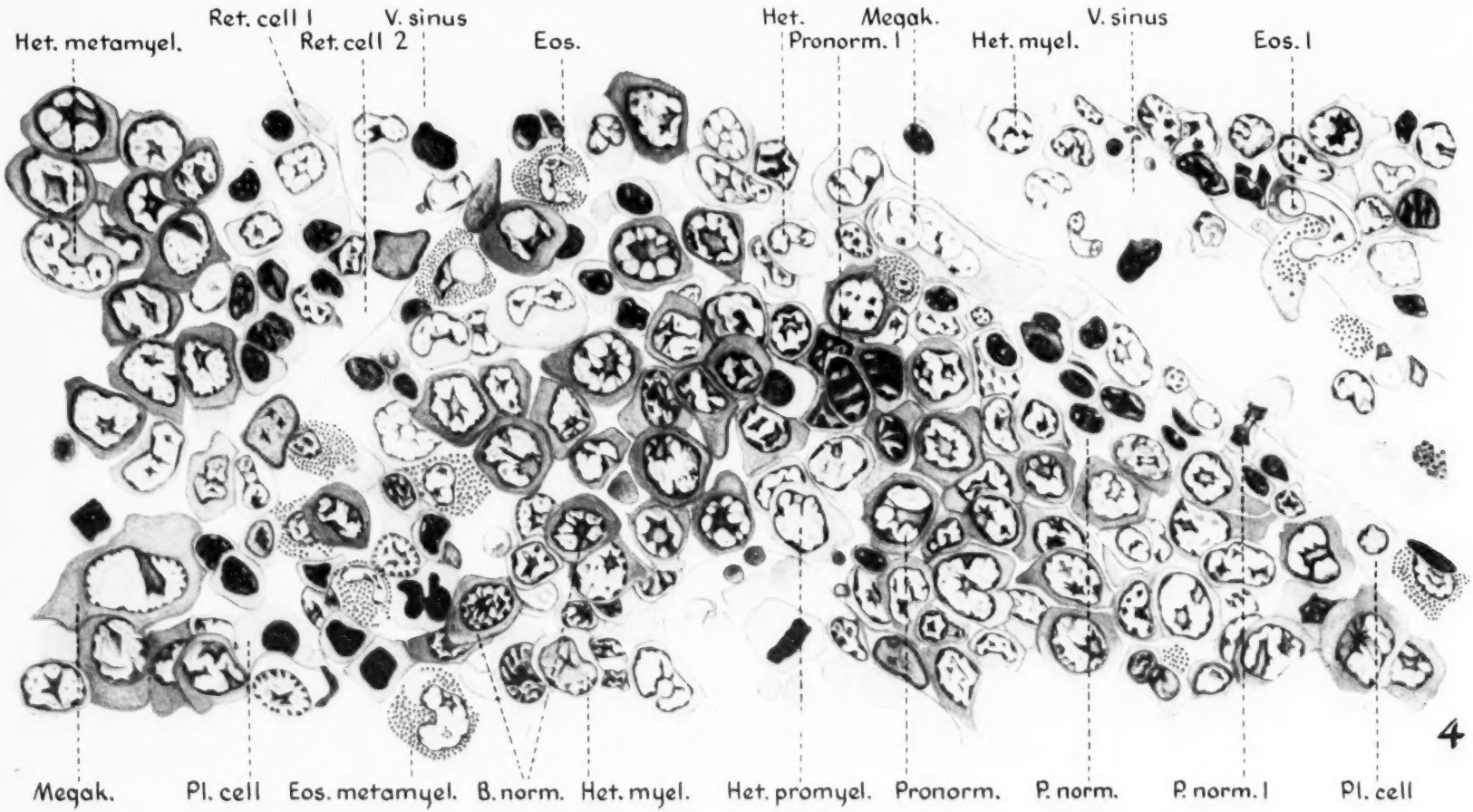


HAMRE: HEMATOPOIESIS IN THE BONE MARROW OF

J. LAB. & CLIN. MED.
VOL. 32, NO. 6, JUNE, 1947.



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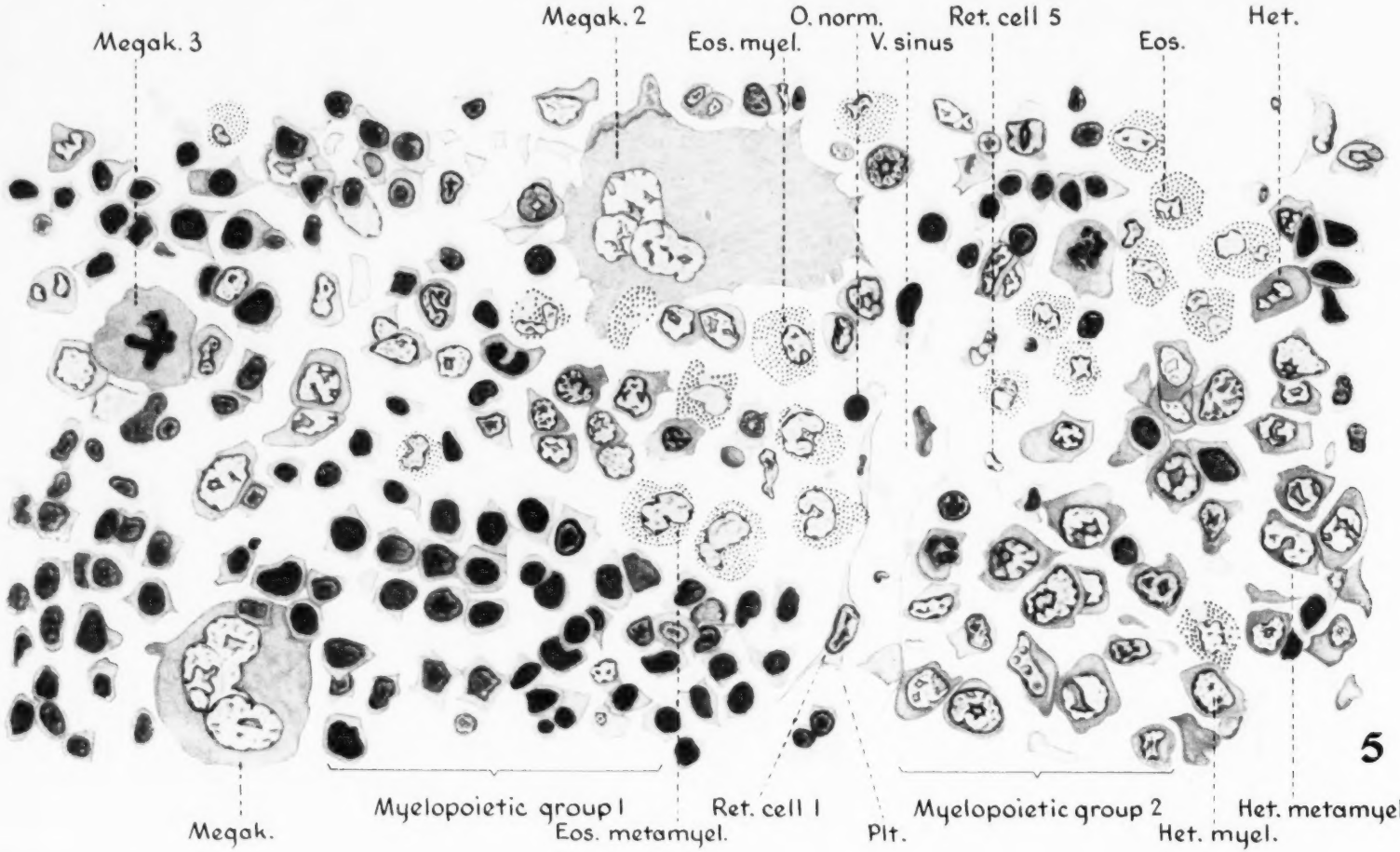

of long processes, basophilia of the cytoplasm, and nucleus with coarse chromatin and distinct chromatin nucleoli, were in the process of changing into hemocytoblasts. The reticular cells at the margin of the intersinusoidal tissue sent processes to the reticular lining cells of the venous sinuses and contributed to the formation of the wall of those vessels. Other reticular cells sent their processes to the walls of the arterioles and arteries located in the stroma. The hematopoietic tissues located in the reticular spaces of the stroma were divided into separate and fairly well-defined groups of differentiating erythrocytes, eosinophiles, and heterophiles. The tendency of the hematopoietic cells to separate into groups of similar cells was best seen in areas of longitudinal sections through the intervascular tissue and the neighboring venous sinuses.

The primary hematopoietic process of the bone marrow of anemic animals was of the homoplastic type. Orthochromatophile and polychromatophile normoblasts, the latter the most numerous, formed the greater part of the nests of erythropoietic cells. It should be emphasized that the cytoplasm of the orthochromatophile normoblasts, as shown in Fig. was not acidophilic as in normal bone marrow but more or less strongly basophilic and stained with the azure stain instead of the eosin stain. They were readily identified, however, by their deeply stained pyknotic nuclei. The basophile character of the cytoplasm of the orthochromatophile normoblasts of the marrow of our anemic animals was expected because many of the erythrocytes of the circulating blood lacked hemoglobin and possessed basophilic or faintly acidophilic cytoplasm. The polychromatophile normoblasts likewise were identified by their nuclei, of coarse, radially arranged and deeply stained chromatin masses and not by the cytoplasm which was basophilic rather than polychromatophilic. Basophile normoblasts were few in number. They were characterized by deeply stained basophilic cytoplasm and a nucleus possessing fairly large chromatin nucleoli and coarse chromatin granules placed on the inner surface of the nuclear membrane. Pronormoblasts were encountered fairly frequently in the nests of erythropoietic cells and differed from the basophile normoblasts in possessing more prominent chromatin nucleoli, finer chromatin granules on the nuclear membrane, and a more irregular cell outline. Mitoses of the erythropoietic cells were very infrequent and it appears that the low erythrocyte count of the anemic animals was the result of arrested cell multiplication and differentiation.

Nests of eosinophilic cells, being almost as numerous as nests of heterophilic cells, were more numerous than the number of eosinophile leucocytes of the circulating blood indicated should have been present. The granules of the developing eosinophilic cells were large and brilliantly stained by the eosin dye. Mature eosinophiles were identified by the presence of an abundance of eosinophilic granules, a faintly acidophilic cytoplasm, and a nucleus more or less deeply indented to form two nuclear lobes. The chromatin of the nucleus of the eosinophiles included small and medium-sized granules in the interior and granules of irregular size placed on the inner surface of the nuclear membrane. The eosinophile metamyelocytes also possessed numerous granules, faintly acidophilic cytoplasm, and a nucleus only slightly indented or perforated at

the center or toward one side. Formation of the eosinophile metamyelocytes from myelocytes was either by indentation or perforation of the nucleus, the latter method of formation occurring most frequently. The eosinophile myelocytes formed the most common cell of the eosinophile nests. They possessed a slightly basophilic cytoplasm, fairly numerous granules, and a spherical nucleus with chromatin nucleoli and numerous fairly fine chromatin granules on the nuclear membrane. Eosinophile promyelocytes, though rarely encountered, were readily identified by their basophilic cytoplasm, reduced number of granules, and a large spherical nucleus. The chromatin of the nucleus of the promyelocyte included fairly large chromatin nucleoli and fine chromatin granules in the interior and on the nuclear membrane. Hemocytoblasts, or myeloblasts, of the eosinophile nests were not numerous but were more numerous than the promyelocytes. They resembled the pronormoblast in shape, basophile staining of the cytoplasm and in the presence of large irregular chromatin nucleoli in the nucleus. They differed from the pronormoblasts in that the chromatin granules on the nuclear membrane were finer and more numerous than those found on the membrane of the pronormoblast. Mitoses of the myelocytes were very infrequent, and it appears that for the eosinophile series, as for the erythrocyte series, cell multiplication and differentiation was suppressed by the anemia.

The cells of the heterophile series of leucocytes also occurred in irregular nests but were the most difficult to identify because the cytoplasm of even the most mature cells of the series was faintly basophilic and because specific granules were not stained or when stained were so small as to be difficult to see. However, it was possible to identify the various stages of differentiation of the heterophiles by comparing their nuclei with the nuclei of the various differentiation stages of the eosinophiles. Since the structural features of the nuclei of the differentiation stages of the heterophiles are like those of the eosinophiles, descriptions of them will not be repeated here. The heterophile promyelocytes were very difficult to identify because of their close resemblance to hemocytoblasts and because of the absence of specific granules. Myelocytes were fairly numerous and differentiation into metamyelocytes was by nuclear perforation or indentation and, as for eosinophiles, most frequently by the former method. Late heterophile metamyelocytes possessed a slender bandlike nucleus while mature heterophiles possessed two or more nuclear lobes. Mitoses of the myelocytes of this series were very infrequent and multiplication and differentiation of the cells of the heterophile series were also suppressed by the anemia.

Basophiles, or mast cells, were encountered very infrequently in the marrow of any of our animals. Plasma cells were found in the marrow of our anemic animals and were usually irregularly distributed through the tissue. Large-sized heterophiles and eosinophiles, most frequently encountered in the myelocyte and metamyelocyte stages, were found in the marrow of the untreated animals. Megakaryocytes showing platelet formation were not numerous. Naked nuclei of degenerating megakaryocytes were also present. An occasional megakaryocyte

was phagocytic, the phagocytosed cells most frequently being normoblasts readily identified by their small, spherical pyknotic nuclei.

The histologic and cytologic changes occurring in the marrow of anemic animals following the feeding of copper and iron supplements can best be described from marrows which had reached the point of greatest response to the therapy. One of the reactions to therapy was the appearance of mitoses in the cells of erythrocyte, eosinophile, and heterophile series. A second and unusual reaction to the therapy was the appearance of large numbers of pronormoblasts and heteroplastic erythropoiesis. The characteristics of a bone marrow showing that reaction in a high degree of development is illustrated in Fig. 4. The marrow illustrated was from an animal killed 22 hours after supplement feeding. Other marrows, namely, of individuals killed at 18, 20, 24, and 26 hours after feeding, showed approximately the same degree of development of heteroplastic erythropoiesis. The occurrence of well-developed erythropoiesis of that type for that fairly long period of time can be accounted for by individual variation in response to therapeutic treatment. The variation observed can be correlated with a similar variation in the values of normoblasts and leucocytes of the circulating blood shown for the same period of time in the graphs of Figs. 1 and 2. It should be noted that the marrow of other animals killed during this period of time also showed heteroplastic erythropoiesis but that the heteroplastic tissue did not occupy as great an area of the marrow.

The marrow at the height of response to therapy was characterized by greater cellularity and compact appearance of the intersinusoidal parenchyma. Venous sinuses, as for anemic animals, were well developed and prominent and their walls well defined. Arteries were located in the central portion of the intersinusoidal tissue area, and in general the vascular system and the intersinusoidal stroma tissue were the same as those found in the marrow of anemic animals. The intersinusoidal hematopoietic tissues were characterized by the presence in the central portion of the area and, therefore, surrounding the arteries and arterioles of a large number of pronormoblasts. Many of the pronormoblasts were in mitosis or were in preparation for mitosis. To either side of the mass of pronormoblasts, between them and the reticular lining cells of the wall of the venous sinuses, were found megakaryocytes and groups of erythrocytes, eosinophiles, and heterophiles in the later stages of differentiation. It was clearly evident in many preparations that the cells of this region were compressed by the new growth occurring in the central region of the intersinusoidal area. Numerous instances of normoblasts and granulocytes passing through the wall of reticular lining cells into the sinuses were found in these preparations. The constricted character of the cells passing through the membrane emphasizes the fact, also readily seen in the sections, that the reticuloendothelial wall of the venous sinuses is a continuous one and that cells pass between the margins of the reticular lining cells. We have concluded that growth pressure within the intersinusoidal areas of our material assists in the migration of normoblasts from the intersinusoidal tissue into the venous sinuses.

The marrows of animals killed at earlier hours after feeding of copper and iron were studied in an effort to determine the origin of the pronormoblasts

which appear in such large numbers during the period of 18 to 26 hours after supplement feeding. It should be emphasized again that the marrow of anemic animals was hyperplastic but that cell multiplication and differentiation was depressed. It should also be pointed out that the vascular system of the marrow of anemic animals, of animals killed a few hours after supplement feeding, and of animals killed at the highest point of heteroplastic hematopoiesis were identical. The pronormoblasts appeared in the intersinusoidal areas and not in the vascular channels. It was noted earlier that instances of reticular cells of the intersinusoidal areas transforming into hemocytoblasts were found in our preparations of the marrow of anemic animals. Instances of such transformation were also found in the marrow of animals killed a few hours after therapy but instances of transformation were not very numerous. Transformation of reticular cells into hemocytoblasts does not satisfactorily account for the formation of such large numbers of hemocytoblasts. However, marrows of animals killed at 8 to 14 hours show many of the hemocytoblasts and pronormoblasts present to be in mitosis, and it appears that the greater number of pronormoblasts were formed in situ by multiplication of those cells and to a lesser degree by transformation of reticular cells into hemocytoblasts.

The marrows of animals killed at 36 and 48 hours after feeding of the iron and copper supplements illustrated in Fig. 5, show the usual arrangement of the venous sinuses and the intersinusoidal tissues. However, pronormoblasts were present in small numbers only, and it is evident that the heteroplastic erythropoiesis had disappeared as the result of the transformation of the pronormoblasts into the later erythropoietic stages. As a result, homoplastic hemopoiesis had been restored. Polychromatophilic and orthochromatophilic normoblasts, many of the former in mitosis, were very numerous. When the marrow of anemic animals and animals killed 48 hours after therapy were compared, it was found that the erythropoietic tissues were more extensive in the latter than in the former marrows. Nests of eosinophiles and heterophiles in homoplastic development were also present. Small megakaryocytes were fairly numerous and large well-developed megakaryocytes showed active platelet formation. Plasma cells and large eosinophiles and heterophiles were present but few in number. All features of the marrow of animals killed after 36 and 48 hours point to a normal condition of very active homoplastic blood cell formation.

DISCUSSION

The present study on dietary anemia of the rat produced by feeding milk alone confirms earlier work in showing that regeneration of the blood is initiated by the addition of copper and iron to the diet. It supports the work of Hamre and Miller[19, 20] on the spleen and Fitz-Hugh and associates[27] on the bone marrow, in showing that the blood-forming organs respond to copper and iron therapy by increased hematopoietic activity. It further shows that the increased cellularity of the bone marrow of recovering anemic rats fed copper and iron noted but not identified by Fitz-Hugh and associates was due to an increase of homoplastic hematopoietic tissue. The material of this study definitely shows that accelerated homoplastic erythropoiesis is established as early as 36

hours after the initial copper and iron administration. It also shows that the first hematopoietic response to copper and iron therapy is the development of heteroplastic erythropoiesis. The heteroplastic erythropoiesis reaches its highest state of development some time during the period of 18 to 26 hours after adding copper and iron to the milk diet. The early heteroplastic erythropoiesis supplies increased erythropoietic tissue for the homoplastic erythropoiesis established 36 to 48 hours after the supplement feeding.

That the bone marrow of anemic animals does respond rapidly and strongly to effective therapeutic agents has also been indicated by other investigators. Doan and co-workers[35] state that the hematopoietic response of the hypoplastic marrow of the radius and the ulna of starved pigeons occurs only 18 hours after feeding is resumed. At that time differentiation of new fat cells begins and signs of reactivation of erythropoiesis appear. They point out that by 50 hours after feeding a normal appearance of the marrow has been established. Jordan and Johnson[36] also investigated the radial marrow of starved pigeons and state that at 28 hours after feeding is resumed hematopoiesis is established and that after that time the only feature of importance respecting hematopoiesis is that it becomes more intense and widespread. The work of McDonald[37] on the hypoplastic marrow of starved pigeons agrees with the observations of the previously mentioned two groups of investigators as far as the time of appearance of increased erythropoietic activity is concerned. Kandel and LeRoy[38] have indicated that in pernicious anemia in man fourteen and a half hours after liver therapy, the megaloblastic character of the sternal marrow has largely disappeared and has been replaced by an increased amount of normoblastic tissue. Bock and Malamos[39] reporting on their own observations and quoting observations of other European workers also point out that there is a rapid transformation of the marrow of patients with pernicious anemia after liver treatment and state that at 48 hours after first treatment megaloblastic cells are difficult to find while the early stages of the erythro-normoblastic type of cells are very numerous. It is obvious that the initial response of the bone marrow of the patients examined by the last group of authors occurred at some earlier hour of the first two days of recovery. Other papers pointing to the time of response of the bone marrow of animals to therapeutic treatment may exist in the literature on anemia but those cited previously in this paper support our view that a "critical period" of erythropoiesis occurs during the first few hours after effective therapy of anemia. For our anemic rats the critical period of recovery extended from 18 to 26 hours after the initial feeding of copper and iron and was characterized by a high degree of development of heteroplastic erythropoiesis. The fact that the height of heteroplastic erythropoiesis did not occur at some one hour of that period for all animals can be explained by the commonly accepted fact of existence of individual differences in the state of a disease and individual differences in reaction to therapy.

The studies of the changes of the blood values of rats recovering from anemia carried out in this investigation show that increases of values for normoblasts, eosinophiles, and heterophiles occur during a period of time which coincides with the critical period of hematopoiesis of the marrow. This period

for those blood cells extends from 12 to 26 hours after therapy. The coinciding of the periods of change of blood values and of the histology of the bone marrow suggest that the two are related and our material shows that the development of heteroplastic erythropoietic tissue is accompanied by a discharge of those blood cells into the venous sinuses. Hamre and Miller,[20] in studying the regeneration of the blood elements of anemic rats following copper and iron feeding, point out that for normoblasts and total number of leucocytes, chiefly heterophiles, the highest values occur on the second and the fifth or sixth day of recovery. Our material suggests that the high values for the second day are the result of the development of heteroplastic erythropoietic tissue in the marrow and the coincident discharge of cells present at the time treatment was begun. We suggest that the second period of high values occurring on the fifth or sixth day of recovery may mark the beginning discharge of a new generation of cells produced from the heteroplastic tissue of the critical period of hematopoiesis. Investigation of the marrow of animals in the second to the fifth day of recovery is needed to establish the significance of the second period of high values of normoblasts and leucocytes.

The marrows of the animals of this investigation, since they possessed large quantities of heteroplastic erythropoietic tissue, form material which bears on the controversial problem of the place within the marrow in which erythrocytes originate and differentiate. The literature bearing on this problem has been reviewed by Doan and co-workers[35] and Sabin and Miller,[40] and a review of the literature will not be presented here. Two views have been advanced in the literature on hematopoiesis, the first view that granulocytes develop extravascularly while erythrocytes develop intravascularly and the second view that both granulocytes and erythrocytes develop extravascularly. Differences of opinion on the place of differentiation of blood cells in the bone marrow, therefore, center primarily about the place of differentiation of erythrocytes; it should be noted that the differences of opinion apply to erythropoiesis in the bone marrow of adult animals since the work of recent authors, Maximow[41] and Bloom and Bartelmez[42] on erythropoiesis in mammalian and human embryos and Dantchakoff[43] and Sabin[44] on avian embryos, agree in describing erythropoiesis as occurring intravascularly. The first view of erythropoiesis, therefore, holds that the embryonic type of intravascular erythropoiesis is continued in the marrow of the adult animal, while the second view holds that embryonic intravascular erythropoiesis during development is lost and is replaced by a new extravascular erythropoiesis in the bone marrow of the adult animal.

The more recent interpretations of the view that erythropoiesis occurs intravascularly in the marrow of adult animals is to a great extent based on the studies of Doan and co-workers[35] on the regeneration of the hypoplastic marrow of starved pigeons after normal feeding was resumed. They concluded that granulocytes are produced in the intervascular stroma but that erythrocytes are produced from the endothelium of hematopoietic capillaries, called intersinusoidal capillaries, originally described by Doan.[45] After maturing within the capillaries, the erythrocytes were described as being carried directly into the circulation and the capillaries as opening to the circulation

and temporarily ceasing erythropoiesis. Jordan and Johnson[36] and McDonald[37] after repeating the studies of these authors[35, 45] doubt or deny the existence of intersinusoidal capillaries in the number or type described and suggest that many of the spaces so labeled are stromal reticular spaces. The latter authors[36, 37] agree that granulocytes take origin in stromal spaces not open to the circulation. They also hold that cells of the erythrocyte series may mature within vascular channels but that the stem cells take origin from the extravascular stroma and then migrate into the vessels for maturation. McDonald also states that some of the erythropoietic cells of the vessels may take origin directly from the endothelium of the vessels without passing through the myeloblast stage. The recent literature on erythropoiesis in the bone marrow of birds indicates that differentiation, and possibly also origin, of erythrocytes occurs intravascularly.

Doan and co-workers[35] have also reported that intersinusoidal capillaries, similar to those described for the marrow of the pigeon, occur in the bone marrow of rabbits. They, therefore, reported erythropoiesis in the rabbit to be intravascular. Peabody[46] claimed to have seen intersinusoidal capillaries in the marrow of man and described erythropoiesis as occurring within them. However, the generally accepted view of hematopoiesis in the marrow of mammals, as noted by Bloom,[47] is that both leucopoiesis and erythropoiesis are extravascular. The generally accepted view of extravascular hematopoiesis is based largely on the clear and detailed descriptions of the process and the histology of the bone marrow of mammals given by Maximow.[48] The work of Drinker and associates[49] and Bunting[50] on the marrow of dogs, cats, and rabbits does not support the idea of the existence of intersinusoidal erythrocytogenic vessels but does support the view that in mammals granulocyte and erythrocyte formation is extravascular.

The review of recent investigations of hematopoiesis in the marrow of birds and mammals gives the impression that granulocytopoiesis is extravascular in both but that they may differ with respect to erythropoiesis. We do not deny the possibility of intravascular erythropoiesis in birds but on the basis of the evidences presented in the literature conclude that it may occur. On the other hand, we feel that evidences presented in the literature point to extravascular erythropoiesis in mammals. Our material on the marrow of the rat, in that it did not show erythrocytogenic vessels comparable to the so-called intersinusoidal capillaries or a change in the pattern of the vascular system during the initiation and development of erythropoiesis, supports that view. We feel that the appearance of new heteroplastic erythropoietic tissue in the parenchyma of the marrow, absence of masses of differentiating erythrocytes from the vascular channels, and the lack of hematopoietic activity on the part of the endothelial lining of the vessels lend strong support to the view that in adult mammals all erythropoiesis is extravascular and localized in the intervascular areas of the marrow.

The heteroplastic erythropoietic marrows of the rats of this study, as pointed out in an earlier section of this paper, supply evidences on the manner of discharge of the inactive erythrocytes from the intersinusoidal areas into the

circulating blood. Explanations of the manner in which erythrocytes are so discharged would differ for instances of intravascular and extravascular erythropoiesis. In the former instance, as pointed out by Doan co-workers,[35] Jordan and Johnson,[36] and McDonald,[37] the erythrocytogenic vessels are continuous with the nonerythrocytogenic vessels and the mature erythrocytes are carried directly into the actively circulating blood of the latter. Explanations of the stimulation of release of the mature cells are not offered. In instances of extravascular erythropoiesis, the endothelial lining cells of the sinuses serve as barriers through which the red blood cells must pass to reach the circulating blood. It is on the possible manner in which this is accomplished that our material yields information.

Drinker and associates[49] have reviewed the literature dealing with the problem of the manner of discharge of red blood cells through the endothelial membrane. They suggest that the endothelial membrane of the sinuses is a continuous one and that groups of erythrocytes expand by growth onto the membrane and due to slight differences in pressure inside and outside the vessel are carried through the membrane. Maximow[48] also held that the membrane is continuous about all of the vessels of the marrow but that over masses of erythrocytes it may temporarily open or rupture to expose the cells to the circulating blood. The plasma passing through the temporary opening loosens the cells and carries them into the circulation. After discharge of the cells the thin endothelial lining membrane closes and the wall of the vessel regains continuity. Key[51] has suggested that the maturation of the erythrocytes is accompanied by a decrease in their cohesiveness and that, as a result, the red blood cells are more readily washed out of the stroma spaces through openings in the endothelial wall into the blood stream. Bunting[50] has suggested that growth pressure may be a factor in causing migration of the red blood cells, though he does not state definitely whether or not he believes the endothelial membrane to be continuous or perforated.

The endothelial membrane of the venous sinuses of our material, as was pointed out previously, is continuous and distinct. Numerous instances of granulocytes and normoblasts passing through the endothelial membrane were found in our material and it appears to us that constriction of both granulocytes and normoblasts at the membrane is significant. We have concluded that the cells pass through the membrane between the margins of the endothelial lining cells and we further have concluded that the intrastromal growth of large quantities of erythropoietic tissue forced the cells through the membrane into the sinuses. We feel that growth pressure is one of the factors, though undoubtedly not the only factor, which brings cells of the erythrocyte series from the intervascular tissue into the blood vessels.

The subject of the origin of the blood cells forms such an extensive mass of publications that it cannot be reviewed here. However, Downey[52] and Cunningham and associates[53] have reviewed the literature and the reader is referred to those publications for detailed information on the subject. It should be noted that the latter authors and Doan and co-workers[35] have advanced the theory that in the bone marrow of adult animals the erythrocyte series takes

origin from the endothelial lining cells of hematopoietic capillaries. Many authors hold that the endothelial lining cells do not possess the potentiality of differentiating into hematopoietic cells and our material supports that view. In our series of animals, the bone marrow was stimulated by copper and iron therapy to produce large quantities of heteroplastic erythropoietic tissue, including large numbers of pronormoblasts. The new tissue was located extravascularly and in none of our preparations did the endothelial lining cells of the sinuses show a change in form or exhibit tendencies toward differentiation of any type.

As was pointed out in the section on results of this study, a special effort was made to determine the source and origin of the pronormoblasts found in such large numbers in our preparations. We did find instances of the reticular cells of the intervascular stroma transforming into hemocytoblasts. Instances of such transformation were not numerous enough to account for the numbers of pronormoblasts produced. However, we did find that after therapy mitosis of the hemocytoblasts was greatly increased. We have concluded that the greater number of pronormoblasts arose by multiplication of hemocytoblasts and pronormoblasts already present at the time therapy was begun or of hemocytoblasts produced by transformation of stromal reticular cells after copper and iron feeding. In none of our preparations were we able to discover cells comparable to the "primitive cell" described by Sabin and associates[54] as the progenitor of granulocytes. Myeloblasts of the type described by Downey,[55] or hemocytoblasts as described by Block,[56] were present in the smears and sections of the bone marrow of our recovering animals. We conclude that they formed the stem cells for granulocytopoiesis and of the pronormoblasts of our preparations.

SUMMARY AND CONCLUSIONS

1. The blood values and bone marrows of sixty anemic rats killed at hourly intervals during the initial 48-hour recovery period after copper and iron treatment were observed in this investigation.

2. Hemoglobin and erythrocytes showed an increase in value beginning at 26 hours after treatment, and a slight but constant increase was continued from that hour to the end of the 48-hour observation period.

3. Normoblasts, eosinophiles, and heterophiles increased in value beginning at 12 hours after treatment, and a period of high and moderately high values extended from that hour to 26 or 28 hours after therapy, this being the period of first discharge of those cells from the bone marrow and the irregularity of values being due to variation in time and extent of response to therapy. Following that period of increase, the values for the various leucocytes continued higher than normal to the end of the period of observation.

4. The first histologic response of the bone marrow to copper and iron treatment was the development of heteroplastic erythropoietic tissue, including numerous pronormoblasts, and the highest degree of development of the tissue was found in the marrows of animals killed 18 to 26 hours after therapy. This period we have called the "critical period" of hematopoiesis of recovery.

5. The period of first great increase in numbers of normoblasts, eosinophiles and heterophiles coincided with critical period of hematopoiesis of the bone marrow and our histologic preparations showed a discharge of those cells into the blood stream by the bone marrow to occur during that period.

6. Erythropoiesis after the critical period was characterized by the disappearance of the heteroplastic process and the development of the homoplastic process, which was continued in increased volume to the end of our period of observation.

7. Erythropoiesis, including the heteroplastic erythropoiesis, and leucopoiesis developed extravascularly in the intervascular stroma; our observations, therefore, support the view that all leucopoiesis and erythropoiesis in the marrow of mammals is extravascular.

8. The lining membrane of the sinuses of our preparations of the marrows was continuous and distinct and there was no rupture or destruction of the membrane during discharge of cells from the intersinusoidal tissues into the sinuses.

9. The development of large quantities of new hematopoietic tissue in the intervascular stroma, and the coincident discharge of cells into the sinuses, suggests that one of the factors concerned in the discharge of cells by the marrow is that of growth pressure within the intervascular stroma.

10. Origin of the pronormoblasts of the new erythropoietic tissue in our preparations was not, as suggested by some authors, from the vascular endothelium but from stromal reticular cells and by multiplication of hemocytoblasts and pronormoblasts. "Primitive cell" progenitors of granulocytes, as described by Sabin and associates, was not recognized in our preparations, and we have concluded that the hemocytoblasts, or myeloblasts, quite numerous in our preparations, were the progenitors of the granulocytes.

The author wishes to express his appreciation to Dr. Hal Downey and Dr. W. Bloom for their interest and suggestions.

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LYMPHOCYTOGENESIS IN HUMAN LYMPH NODES

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THE origin, the development, and the widely varied morphologic changes, which are manifest during lymphocytomorphosis, have been and still are some of the most fundamental problems in all hematology. Classifications of the various types of cells grouped under the term lymphocyte are notoriously unsatisfactory, because lymphocytes lack the easily demonstrable specific characteristics such as the specific granules of granulocytes or the hemoglobin of developing erythrocytes. Although this lack of convincingly identifiable cytologic landmarks must be accepted as inevitable, the recent experiments showing that lymphocytes contain a protein identical with normal serum gamma globulin[1, 2] and that antibodies are present in the lymphocytes of immunized animals[3, 4] are dramatic examples of the importance of lymphocytes, examples which illustrate the need for renewed efforts at a clearer understanding of lymphocytomorphosis.

The origin of the lymphocyte in lymphatic tissue of laboratory animals and a comparison of the various types of lymphocytes to the myeloblasts of the bone marrow have been the subjects of research on the part of Downey and co-workers.[5-12] From these investigations and from studies of both blood and lymph nodes in infectious mononucleosis[13, 14] and in subacute lymphatic leukemia,[15] it was inferred that lymphocytogenesis in nonleukemic human nodes probably closely simulated that which occurs in normal lymph nodes of rabbits and guinea pigs. The voluminous literature pertaining to the problems involved is partially reviewed in most of the publications but is most complete in the latest articles.[11, 12]

The present report is a summary of a study of lymphocytogenesis in human lymph nodes. The various cell types involved were compared with the myeloblast of human bone marrow, with the immature and mature lymphocytes of the lymph nodes of rabbits and guinea pigs, and with the types of lymphoid cells seen in the lymph nodes, blood, and bone marrow of cases of infectious mononucleosis, chronic lymphatic leukemia, and acute and subacute lymphatic leukemias.

MATERIALS AND METHODS

The 55 human lymph nodes studied were surgical specimens from which imprint preparations were made within a few minutes after their removal. They included 43 which showed no evidence of lymphoblastoma, 2 from chronic lymphatic leukemia, 9 from infectious mononucleosis (including the 8 nodes studied by Downey and Stasney[14]), and 1 from subacute lymphatic leukemia (that described by Stasney and Downey[15]). Eleven lymph nodes from rabbits and guinea pigs were useful in comparative studies; they have been described elsewhere.[12]

The 43 nonleukemic nodes included nodes from the lesser curvature of the stomach (4), the inguinal region (10), the axilla (24), and other locations (5). In all but four of the cases

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in which the nodes showed no evidence of leukemia, the patients' clinical and hematologic findings were investigated and found to be within the range of those expected with the varied causes of hospitalization. In no case was there any evidence of leukemia.

Direct smears of bone marrow and smears made of the myeloid-erythroid layer obtained from heparinized centrifuged bone marrow[16] from a wide variety of conditions other than leukemia were also examined in order that the various morphologic variations of the myeloblast might be correlated with the variations in the least differentiated types of lymphocytes visible in the nodes.

During the course of this investigation, blood and bone marrow received for diagnosis was also considered critically with reference to the various types of immature lymphoid cells encountered. The pathologic material reviewed which helped clarify the present problem included: (1) 50 cases of chronic lymphatic leukemia: blood, 34; blood and bone marrow, 16. (2) 31 cases of subacute or acute lymphatic leukemia: blood, 21; blood and bone marrow, 10. (3) 34 cases of infectious mononucleosis: blood, 33; blood and bone marrow, 1. (4) 12 cases of brucellosis: blood, 3; blood and bone marrow, 9.[17]

In order to determine the possible influence of blood on the cells from lymph nodes, a small amount of material from the mesenteric lymph node of a rabbit was scraped from a cut surface of the node and mixed with a small amount of blood. Smears were made from this mixture.

Lymph node imprints and smears of blood and bone marrow were stained with either the May-Grünwald-Giemsa combination or with Wright's stain. Lymph nodes were fixed in Helly's fluid, cut at 5 micra, and stained with hematoxylin and eosin or with Dominici's stain.

OBSERVATIONS

Lymph Node Sections.—Photomicrographs of sections of the lymph nodes have not been included because the development of lymphocytes from reticular cells has already been exhaustively studied by earlier investigators. The following works are singled out because they best illustrate the problem in question here. Downey and Weidenreich[5] described and included beautiful illustrations (their Figs. 2 and 3) of the transformation of fixed reticular cells to lymphocytes in sections of guinea pig lymph nodes. Thiel and Downey[18] described and illustrated the transformation of mesenchymal cells to hemocytoblasts and lymphocytes in the spleen of the pig embryo. Maximow[19] gave excellent illustrations of the types of cells seen in germ centers of rabbit (his Figs. 7 and 8) and human (his Figs. 9 and 10) lymph nodes and in a germ center of human tonsil (his Fig. 11). Maximow also showed the formation of large lymphocytes from the embryonic reticular syncytium in a newly formed lymph nodule in the mesentery of a rabbit (his Fig. 12). Klemperer[20] described and illustrated the transition from fixed reticular cells to hemocytoblasts in human pathologic lymph nodes and spleen.

Histopathology of Nonleukemic Nodes.—Since the development of lymphocytes rather than the types of pathologic changes occurring in lymph nodes is the subject of this report, the histopathologic changes observed in the lymph nodes can be considered only very briefly. None of the nodes examined was completely normal. Two nodes showed changes which might have resulted from mild physiologic (?) inflammation or age. Imprints from these nodes showed no evidence of active regeneration of lymphocytes. Five nodes which showed evidence of acute lymphadenitis were more normal in structure than any of the other specimens. Two of these nodes contained a fairly large number of small

germinal centers, and imprints showed an increase in the number of developing lymphocytes. Thirty-four nodes showed evidence of simple hyperplasia accompanied by chronic inflammatory changes. Of these thirty-four nodes, twenty-six were draining carcinomata, but only four of the latter showed the presence of tumor cells. The thirty-four simple hyperplastic nodes were those best adapted to this problem in that they all, regardless of the precipitating cause of hyperplasia, contained germinal centers in various stages of activity and showed increased numbers of developing lymphocytes in imprint preparations. Particularly interesting was the fact that the nodes draining the carcinomatous lesions showed pronounced lymphoid hyperplasia and many very large germinal centers, some of which were comparable in size to those seen in follicular lymphoblastoma. Only a very small number of macrophages containing the tingible Körper of Flemming[21] were seen in the large number of these centers which were studied. The centers were not reaction centers in the proper sense; they were actively engaged in the production of lymphocytes, for they were filled with reticular cells and transitional stages between reticular cells and lymphocytes, and mitoses were numerous. Because some of the imprints from these nodes contained large aggregates of lymphoid cells including primarily reticular cells, reticular lymphocytes, and cells in various stages of mitosis, it was concluded that actual *germinal centers had been imprinted*, and the comparison of the immature cells in sections and in imprints could be made with considerable assurance.

Nine nodes, biopsy specimens from cases of infectious mononucleosis, showed the histopathologic alterations ascribed to this disease,[14] and the imprints and smears showed a great increase in both the numbers of hematopoietic reticular cells and reticular lymphocytes[22] and of leukocytoid lymphocytes of various types.[13]

Transformation of Reticular Cells to Lymphocytes.—It is recognized that undifferentiated, primitive, fixed, or syncytial reticular cells are capable of transforming either to phagocytic cells or to hematopoietic cells. In sections of the lymph nodes, reticular cells, even while still in syncytial arrangement, could be seen to have undergone partial differentiation. The differentiation to phagocytic cells could be recognized by the presence of slight coarsening of the nuclear chromatin and increased vacuolization of the cytoplasm as well as by the presence of ingested particles. The differentiation to lymphocytes was manifested both by coarsening of nuclear chromatin and by increased basophilia of the cytoplasm. Although these changes could be seen in fixed or syncytially arranged reticular cells, more often the differentiating reticular cells were free. In imprint preparations, undifferentiated reticular cells which were apparently free could also be found (Fig. 1).

The transformation of undifferentiated reticular cells to lymphocytes as it occurs in human lymph nodes was found to be essentially similar to that previously described for the nodes of rabbits and guinea pigs.[12] The transformation was a gradual process involving many morphologic cell types which differed but slightly from one another. The stages in the transformation appeared to include (1) undifferentiated reticular cells, (2) hematopoietic reticular cells,

(3) reticular lymphocytes, and (4) lymphocytes identical with those of the blood. Mitoses were found to be most numerous in cells which, because of their size and the character of their cytoplasm (Fig. 11), are believed to be hematopoietic reticular cells and reticular lymphocytes rather than the common large lymphocytes of the blood. No cells morphologically identical with the myeloblast (Figs. 14, 14′, 15) of the bone marrow or with the lymphoblast (Figs. 16, 16′, 17) of acute and subacute lymphatic leukemias were found to participate in the regeneration of lymphocytes in the nonleukemic nodes.

The undifferentiated reticular cell (Fig. 1) is a large cell which possesses a relatively large amount of cytoplasm. Its nucleus, like those of the lymphoid cells derived from it, is generally round or oval in shape. The nuclear chromatin is fine in character, stains a light reddish violet, and is distinctly demarcated from the pale pink-staining and relatively abundant parachromatin (karyoplasm). The chromatin pattern is delicate. In areas, it appears to be net- or sievelike; however, generally small clumps, chains, and strands of chromatin are also present, and the resulting nuclear pattern is not uniform as is that of most myeloblasts. Nucleoli may or may not be present. They are variable in size but are usually small blue-staining bodies which are partially obscured by overlying chromatin. The nuclear membrane is fine but distinct.

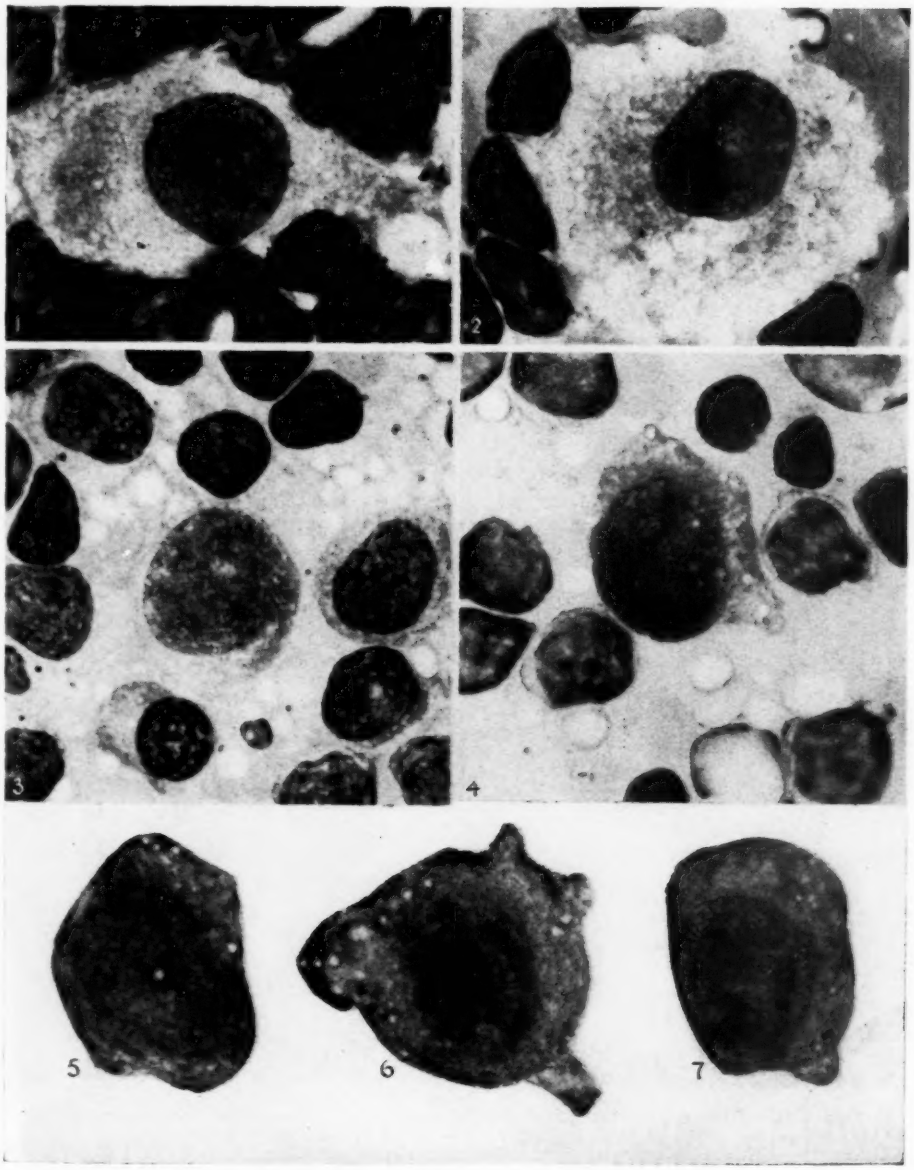
The cytoplasm of imprinted undifferentiated reticular cells is frequently damaged (Fig. 1); its actual boundaries are often not clearly visible. It appears to be watery, consisting of an abundant hyaloplasm with only a small amount of faintly basophilic spongioplasm. Vacuoles may be present; azure granules also can sometimes be found.

Figs. 2 and 2′ show a cell very similar to the undifferentiated reticular cell. It has distinct cytoplasmic boundaries. Cells, not illustrated here, which occurred in the fields surrounding this cell contained phagocytosed material. Although the reticular cell in these figures might be considered an undifferentiated cell, in all probability it is a potential phagocyte (histiocyte). It is included in order best to illustrate the type of aggregation of chromatin which can be seen in the nuclei of reticular cells. (Compare the nuclear pattern of this cell with that of the hematopoietic reticular cell in Figs. 7 and 7′.)

In the transformation of the undifferentiated reticular cell to a one capable of forming blood cells, the most conspicuous change is a decrease in the amount of cytoplasm. The cytoplasm of hematopoietic reticular cells may be sparse (Figs. 3 and 4) but usually it is abundant (Figs. 5, 6, 7, 7′). Although basophilia of the cytoplasm is variable, most of the hematopoietic reticular cells have a grey-blue cytoplasm which is generally mottled or flaky in appearance and which often contains vacuoles (Figs. 3, 4, 5, 6, 7, 7′). The nuclear pattern may be almost identical with that of the undifferentiated reticular cell. (Compare nuclei of cells in Figs. 1 and 3.) The various cells classified as hematopoietic reticular cells because they resemble undifferentiated reticular cells and do not show indisputable evidence of transforming to lymphocytes are shown in Figs. 3, 4, 5, 6, 7, and 7′.

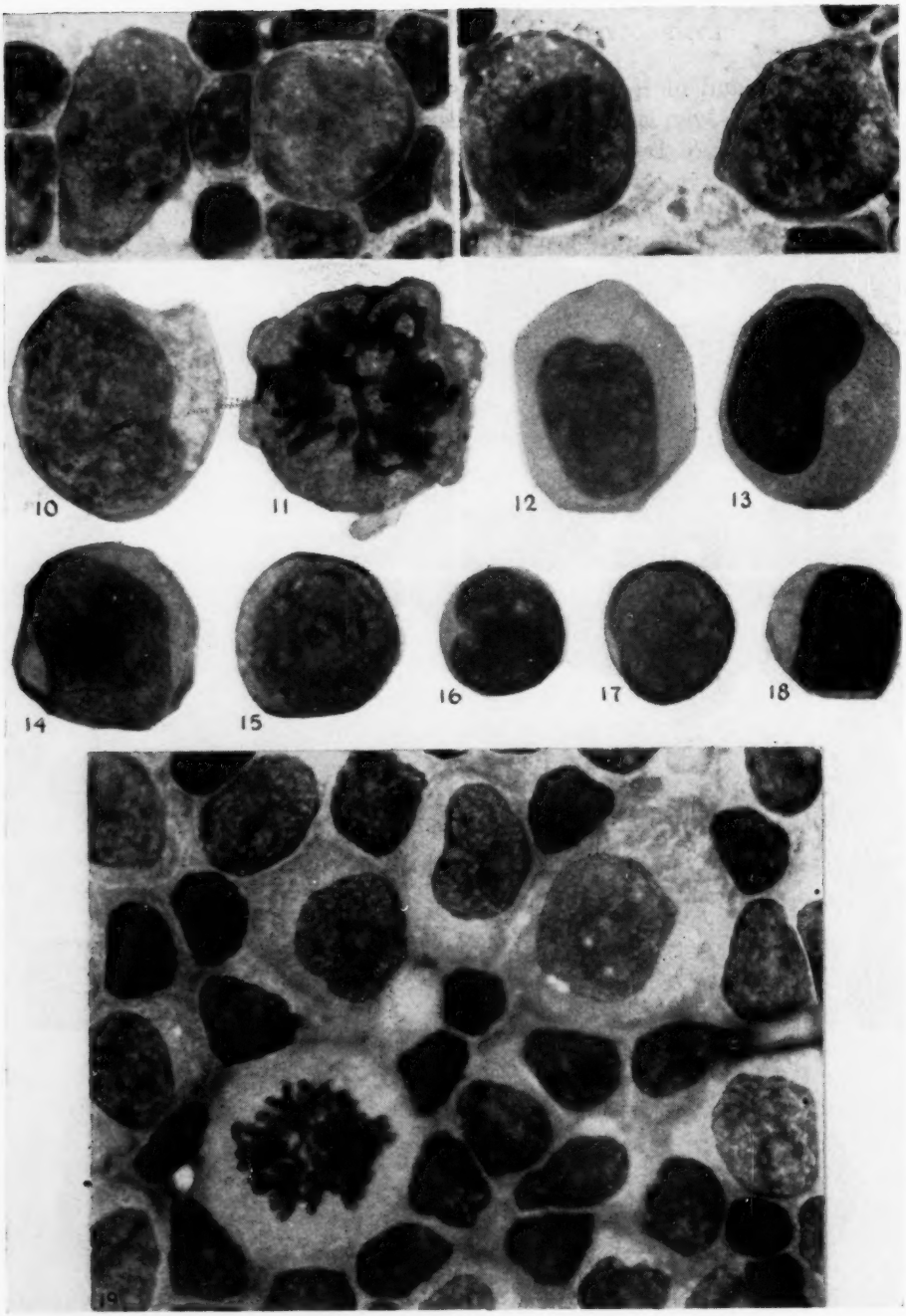
The next classifiable stage of differentiation is the reticular lymphocyte (Figs. 8 to 10). This cell has morphologic characteristics similar to those of

reticular cells and of lymphocytes. The recognition of this cell type as one distinct from the lymphoblasts of acute leukemia is of prime importance because small percentages of reticular lymphocytes may occur in the peripheral blood (Figs. 12, 12′, 13) in benign lymphatic reactions.



Figs. 1-7.—Cell types occurring in imprints of human nonleukemic lymph nodes which showed variable degrees of hyperplasia. All photomicrographs taken at same magnification (×1440).

1.—Undifferentiated reticular cell.
2.—Histiocytic reticular cell.
3.—Hematopoietic reticular cell. Lymphocytes. Plasma cell.
4-7.—Hematopoietic reticular cells.



Figs. 8-10.—Reticular lymphocytes from imprints of hyperplastic human lymph nodes (×1440).

Fig. 11.—Mitosis in hematopoietic reticular cell or reticular lymphocyte (×1440). Imprint of hyperplastic human lymph node.

Figs. 12 and 13.—Reticular lymphocytes (×1440) from blood in cases of infectious mononucleosis.

Figs. 14 and 15.—Myeloblasts (×1440) from nonleukemic human bone marrow.

Figs. 16 and 17.—Lymphoblasts (×1440) from blood in cases of acute lymphatic leukemia.

Fig. 18.—Lymphocyte (×1440). Imprint of hyperplastic human lymph node.

Fig. 19.—Imprint of lymph node from chronic lymphatic leukemia. Hematopoietic reticular cells, reticular lymphocytes, lymphocytes, and mitosis (×1440).

Narrow-bodied cells similar to the cell at the right in Fig. 8 were most similar to the myeloblasts of the marrow and to the lymphoblasts of acute and subacute lymphatic leukemias. These narrow-bodied immature lymphoid cells differ from lymphoblasts in that considerable aggregation of chromatin is present. However, cells of this type may be encountered in the peripheral blood in acute and subacute lymphatic leukemias. It is assumed that these cells represent immature lymphocytes, but their phase of maturity is such that it is not possible to determine whether they have been derived from reticular cells or from lymphoblasts.

The stages occurring during the transition of reticular lymphocytes to cells which exhibit the characteristic morphology of the normal lymphocytes of the blood are numerous. The changes include a diminution in the cell size and in the size of both nucleus and cytoplasm. The nuclear chromatin becomes coarse and is arranged in heavy clumps of variable size. The latter stain deeply and tend to blend with the relatively sparse parachromatin. The nuclear membrane is coarse and distinct; nucleoli are not visible. The cytoplasm is colorless or pale blue and more hyaline in appearance. (Note lymphocytes in Figs. 18 and 3.)

Comparison to Myeloblasts.—As has been stated, no isomorphs of the myeloblasts of human bone marrow were found in the imprints of human nonleukemic lymph nodes. There is, as is commonly recognized, a marked similarity

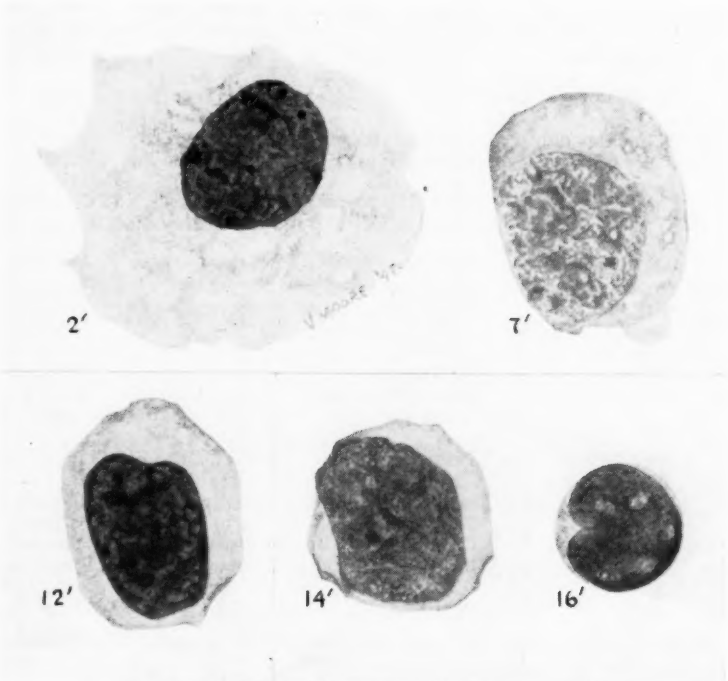


Fig. 2′.—Histiocytic reticular cell, same as Fig. 2.
Fig. 7′.—Hematopoietic reticular cell, same as Fig. 7.
Fig. 12′.—Reticular lymphocyte, same as Fig. 12.
Fig. 14′.—Myeloblast, same as Fig. 14.
Fig. 16′.—Lymphoblsat, same as Fig. 16.
(Drawn from original cells with the aid of photomicrographs. Magnification, ×1440.)

between blast forms of all types, and the morphologic characteristics which distinguish the hematopoietic reticular cell from the myeloblast are neither numerous nor prominent. Since both myeloblasts and lymphoblasts may be derived from reticular cells, morphologic evidence of the reticular derivation of either of these cells is sometimes present. The uniformity of nuclear structure in both myeloblasts and lymphoblasts might well be the result of repeated mitoses without subsequent differentiation.

Table I is included in order to facilitate comparison of the morphologic characteristics most commonly encountered in hematopoietic reticular cells, reticular lymphocytes, large lymphocytes, and myeloblasts or lymphoblasts. These characteristics are, of course, simply average findings.

TABLE I. COMPARISON OF IMMATURE LYMPHOCYTES WITH LARGE LYMPHOCYTE AND MYELOBLAST

Cell type	Hematopoietic reticular cell	Reticular lymphocyte	Large lymphocyte	Myeloblast or lymphoblast
Cell size	23 by 19 micra	20 by 17 micra	14 by 12 micra	16 by 15 micra
Nuclear size	17 by 14 micra	16 by 13 micra	11 by 9 micra	14 by 13 micra
Nuclear shape	Round or oval	Round or oval	Round or oval	May be irregular
Nuclear pattern	Irregular	Irregular	Irregular	Uniform
Chromatin				
Staining	Light reddish violet	Reddish violet	Purple	Light reddish violet
Nature	Small particles of variable size; chains; strands of granules	Similar to hematopoietic reticular cell but larger masses; more strands of granules	Large angular masses; only very few chains	Fine, sieve- or netlike; sometimes granular or stippled
Parachromatin				
Amount and nature	Abundant and very distinct	Abundant and distinct	Sparse and indistinct	Not overly abundant but distinct
Staining	Pale pink	Pale pink to pale reddish violet	Pale reddish violet to gray blue	Pale pink
Nucleoli				
Number	Variable	Variable	Not visible	Variable
Diameter	1 to 7 micra	1 to 7 micra		1 to 3 micra
Staining	Pale blue	Pale blue to blue		Pale blue
Shape	Rounded or irregular	Rounded or irregular		Round or oval
Character	Indistinct	Indistinct		Distinct
Nuclear membrane	Fine, distinct	Coarse, less distinct	Coarser, less distinct	Very fine, indistinct
Cytoplasm				
Staining	Gray blue to colorless	Deep blue to colorless	Pale blue to colorless	Pale blue
Character	Mottled and flaky	Mottled and flaky	Usually homogeneous	Usually homogeneous
Vacuoles	Common	Common	Uncommon	Uncommon
Azure granules	Rare	Rare	Uncommon	Common in myeloblast; uncommon in lymphoblast

Lymphocytogenesis in Animal Lymph Nodes.—Lymphocytogenesis in human lymph nodes was found to be remarkably similar to lymphocytogenesis in the nodes of rabbits and guinea pigs. In the hyperplastic but nonleukemic human nodes, hematopoietic reticular cells were, however, more numerous than

in the nodes of rabbits and guinea pigs. In the nodes of rabbits and guinea pigs, reticular lymphocytes, most of which possessed a more deeply basophilic cytoplasm than did the reticular lymphocytes of human nodes, were far more numerous than hematopoietic reticular cells, and mitoses were most numerous in these reticular lymphocytes. No important morphologic changes were produced by mixing the cells from a rabbit's lymph node with blood.

Lymphocytogenesis in Infectious Mononucleosis.—Lymphocytogenesis in infectious mononucleosis resembled that seen in both the simple hyperplastic human nodes and the animal nodes. As would be expected, however, there was more evidence of active regeneration of lymphocytes in the nodes from infectious mononucleosis than in any of the other nonleukemic nodes. The following features were recognized: (1) Hematopoietic reticular cells and reticular lymphocytes were more numerous in many of the lymph nodes from infectious mononucleosis than in most of the other nonleukemic nodes, and reticular lymphocytes were far more numerous than hematopoietic reticular cells. (2) The cytoplasm of the immature cells seen in infectious mononucleosis generally showed pronounced basophilia, and in many of these cells the cytoplasm was almost completely homogeneous. (3) Some of the extremely basophilic reticular lymphocytes appeared to function as precursors of plasma cells. However, transitions between these basophilic reticular lymphocytes (plasmablasts of Moeschlin[23, 24]) and the Type I leukocytoid lymphocyte of Downey and McKinlay[13] were also prominent. (4) Mitoses were more numerous in many of the lymph nodes from infectious mononucleosis than in any of the other nonleukemic nodes. Many more mitoses appeared to occur in the reticular lymphocytes of the lymph nodes of infectious mononucleosis than in the reticular lymphocytes of the other nonleukemic nodes. (5) In the lymph nodes from infectious mononucleosis, transitions between the reticular lymphocytes and the leukocytoid lymphocytes of infectious mononucleosis were numerous, whereas such transitions were very rare in the other nonleukemic nodes.

The bone marrow from one case of infectious mononucleosis showed a relative (29.5 per cent) and absolute lymphocytosis (myeloid-erythroid volume, 18.5 per cent). Hematopoietic reticular cells, reticular lymphocytes, and leukocytoid lymphocytes were numerous. All three of these cell types were morphologically distinct from the myeloblasts of this marrow.

Lymphocytogenesis in Chronic Lymphatic Leukemia.—Lymph nodes from two cases which exhibited the typical clinical and hematologic findings of chronic lymphatic leukemia were studied. The node from the first case had been palpable for a long period of time; the node from the second case had become noticeable in a period of about three weeks. The former node was considered to be relatively static, and it will be designated Node S; the latter was one which had undergone rapid growth, and it will be designated Node G. Each of these nodes showed in section preparations the characteristic features of chronic lymphatic leukemia. In Node G, however, reticular hyperplasia and mitoses were more prominent.

The imprint material from Nodes S and G showed such a striking lack of similarity that the relatively similar appearing sections from these two nodes seemed almost incomprehensible.

Node S showed an almost completely monotonous picture of large lymphocytes. These lymphocytes had a nuclear pattern only slightly different from that of the large lymphocytes of normal blood. The chromatin masses were neither as heavy nor as deeply stained as those of ordinary lymphocytes, and the parachromatin was more clearly visible than is that of normal lymphocytes. The cytoplasm was less abundant than that of most lymphocytes, but it was not as sparse as that of the lymphoblasts of acute lymphatic leukemia or as that of many of the myeloblasts of human nonleukemic bone marrow. The most prominent feature was not, however, this slightly abnormal appearance of the lymphocytes, for lymphocytes of this type can be found in almost any lymph node imprint. The striking feature was the lack of variation in cell type. Cells which appeared less differentiated than these large lymphocytes were present, but they were neither particularly numerous nor conspicuous. Some resembled the hematopoietic reticular cells and reticular lymphocytes of nonleukemic nodes, but most of them showed an even more striking clarity of nuclear detail than that seen in similar cells from nonleukemic nodes. Nucleoli, particularly, seemed more distinct. Yet, none of the cells seen was identical with the myeloblast (Figs. 14, 14′ 15) of the bone marrow or with the lymphoblast (Figs. 16, 16′, 17) of acute lymphatic leukemia. All of the immature cells showed morphologic patterns interpreted as being indicative of their close relationship to reticular cells.

Node G, in contrast, showed a picture which was completely lacking in the monotonous uniformity of cell type which was seen in Node S. Although all sizes of lymphocytes were present, the lymphocytic picture was overshadowed by tremendous numbers of cells many of which were almost identical to the hematopoietic reticular cells and reticular lymphocytes that occurred in the nonleukemic nodes. (Note, for example, the striking similarity of the largest cell shown in Fig. 19 to the hematopoietic reticular cell shown in Figs. 7 and 7′. Compare also the large cells in Figs. 5 and 19; both have large and irregularly shaped, indistinct nucleoli, and both have vacuoles in both nuclei and cytoplasm. Finally, compare the mitotic figure in Fig. 19 with that in Fig. 11.) Many cells which were morphologically identical with the hematopoietic reticular cell in Figs. 7 and 7′ were present in Node G. Everywhere throughout all of the imprints of Node G hematopoietic reticular cells, along with intermediate stages between them and lymphocytes, and mitoses were numerous. In fact, one might compare the picture as a whole to one which could be produced by imprinting one mammoth germinal center. This degree of hyperplasia was not, however, encountered in any of the nonleukemic human or animal nodes. In imprints from Node G, as in those from Node S, there was a striking clarity of cytologic detail. Because it was suspected that this might be caused by some variation in technique, nonleukemic and leukemic nodes were stained simultaneously and in an identical manner. The resulting preparations showed that the method of staining was not the explanation for this phenomenon.

The degree of reticular hyperplasia which was evident in Node G is considered unusual for chronic lymphatic leukemia. Most of the nodes from chronic lymphatic leukemia which have been described and which are encountered in routine autopsy material show the monotonous uniformity of pattern seen in

Node S of this report. In the case from which Node G was removed, neither the patient's clinical condition nor his hematologic findings suggested an exacerbation of the leukemic process. The best explanation for the active regeneration of lymphocytes seen in the node seemed to be that the node had undergone recent rapid growth.

Lymphocytogenesis in Acute and Subacute Lymphatic Leukemias.—Lymphoblasts from acute lymphatic leukemias in which the peripheral blood smears showed large numbers of cells of this type are shown in Figs. 16, 16′, and 17. These cells are very similar to the cells seen in the imprints of the biopsied lymph nodes studied by Stasney and Downey,[15] and they are also similar to many of the cells seen in the peripheral blood of their case. Since no lymph nodes from acute or subacute lymphatic leukemia were obtained during the course of this study, conclusions were based upon a comparison of the cell types seen in nonleukemic nodes with the lymphoblasts seen in the peripheral blood and in the bone marrow of cases of acute and subacute lymphatic leukemia and with the lymphoblasts seen in the imprints of the nodes studied by Stasney and Downey.

The nonleukemic nodes did not contain cells morphologically identical with the leukemic lymphoblasts. The leukemic nodes and the peripheral blood smears and bone marrow smears from the various cases of acute and subacute lymphatic leukemias contained large numbers of lymphoblasts many of which could not be distinguished from myeloblasts. In addition, the smears from the acute and subacute leukemias also contained occasional hematopoietic reticular cells and reticular lymphocytes as well as immature lymphocytes with narrow cytoplasmic bodies similar to the cell at the right in Fig. 8. The latter type of cell can be found in relatively normal and in hyperplastic nodes, in the blood, nodes, and marrow of lymphatic leukemia, and also occasionally in the blood of normal children.

REVIEW OF LITERATURE AND DISCUSSION

Publications presenting detailed morphologic studies of lymphocytogenesis as seen in both imprint and section preparations of surgically biopsied, human nonleukemic nodes have not been encountered. However, various investigators who have aspirated material from lymph nodes for diagnostic purposes have discussed regeneration of lymphocytes in normal nodes.

Fleischhacker and Klima[25] stated that "In order to get a criterion about the morphology of normal lymph nodes, we punctured patients with good available but otherwise unchanged lymph nodes." They found that smears of these normal nodes showed predominantly ripe lymphocytes, but a few larger, less mature forms were present and were interpreted as lymphoblasts. They made a distinction between myeloblasts of myelogenous leukemia and lymphoblasts, but they made no distinction between the lymphoblasts of normal nodes and those of lymphatic leukemia.

Tischendorf[26] confirmed some of the conclusions of the present study by stating that the puncture of healthy nodes was hardly possible and by adding that when lymph nodes are large enough to be punctured, they are probably pathologic. Tischendorf, however, also described typical lymphoblasts in nodes

and claimed they were increased in number in hyperplasias and in leukemias. He clarified his interpretation of the term lymphoblast by stating that in some leukemias, large cells with spongy nuclei containing intensively blue and distinct nucleoli, and whose protoplasm was delicate and often "fringed out," could be seen. These cells *may*, according to him, be stem cells which may be derived from the capillary endothelium. Of particular interest was the fact that Tischendorf also discussed a case of chronic lymphatic leukemia from which the lymph node material showed large numbers of immature cells (his stem cells and lymphoblasts). In lymph aspirated from the nodes of infectious mononucleosis, he found cells two times the size of lymphocytes with fine nuclei which "lie in a deep dark blue cytoplasm." These cells were regarded as characteristic cells of infectious mononucleosis which showed no resemblance to the immature lymphoid cells of relatively normal nodes. Tischendorf, like Fleischhacker and Klima,[25] considered myeloblasts and lymphoblasts as morphologically distinct cell types.

It is difficult to determine how much agreement there is between the present findings and those of the German authors.[25, 26] In the present study, the term lymphoblast is reserved, as it has been in the preliminary studies of lymphocytogenesis, for the immature lymphoid cell of acute and subacute lymphatic leukemias. The lymphoblast is considered to be a cell which is frequently morphologically indistinguishable from the myeloblast. Fleischhacker and Klima[25] and Tischendorf[26] claimed separate identity for myeloblasts and lymphoblasts, but they found lymphoblasts in normal as well as in leukemic nodes. Although Tischendorf claimed that the immature cells of infectious mononucleosis bore no resemblance to the immature cells of relatively normal nodes, it seems extremely likely that the cells which he observed in infectious mononucleosis would correspond to the hematopoietic reticular cells and reticular lymphocytes of the present report. As has been previously discussed, the immature lymphoid cells of infectious mononucleosis are, except for more intense cytoplasmic basophilia, almost identical to those seen in other hyperplastic nodes.

Moeschlin,[24] in his report on a study of three cases of infectious mononucleosis in which he studied aspiration material from lymph nodes, bone marrow, and spleen as well as the blood, showed that much of his work confirmed the previous work of Downey and Stasney.[14] However, in this paper and in a previous work on lymph node aspiration material from rubeola,[23] Moeschlin described two immature cells which he felt were specific cell types. The first of these cells was the plasmablast, and since he could see transitions between this cell and plasma cells, he felt that it was much more likely that the plasma cells formed in lymphatic tissue were derived from this plasmablast than from lymphocytes. As nearly as can be determined from his descriptions and his Fig. 4, *a*,[23] Moeschlin's plasmablast appears to correspond to some of the unusually basophilic hematopoietic reticular cells and reticular lymphocytes seen in the present investigation.

In view of Moeschlin's hypothesis, however, nodes of all types were studied in an effort to determine whether the large reticular elements should be considered as plasmablasts. Transitions between reticular lymphocytes and plasma cells were encountered, but transitions betweeen lymphocytes and plasma cells

were also often seen in nodes where no cells resembling the plasmablasts of Moeschlin could be found, and cells corresponding well with his plasmablast were found in nodes which contained few or no plasma cells. Therefore, in spite of Moeschlin's excellent figures, it does not seem correct that he designate the extremely immature hematopoietic reticular cell as a specific plasmablast and ignore the fact that this cell may function as a parent cell for all the lymphatic elements of the nodes, including plasma cells. (Note plasma cell in Fig. 3.)

The second immature cell type was Moeschlin's[24] lymphatic monoblast. This cell (his Fig. 5, *1*), which occurred in increased numbers in infectious mononucleosis appears to correspond to the reticular lymphocytes of the present investigation. Moeschlin called it a lymphatic monoblast because transitional stages occurred between it and a lymphatic cell (which showed an indented nucleus and coarser chromatin), the lymphatic monocyte. The latter cell corresponds to Downey and McKinlay's[13] leukocytoid lymphocyte, Type I. Here, again, terminology is confusing. Moeschlin's term lymphatic monoblast is an acceptable descriptive term, for certainly some of the leukocytoid lymphocytes of infectious mononucleosis resemble monocytes and might be called lymphatic monocytes. However, cells entirely similar to Moeschlin's lymphatic monoblasts (reticular lymphocytes of the present investigation) were numerous in nodes which were hyperplastic but which showed almost no lymphocytes with indented nuclei resembling the lymphatic monocytes of Moeschlin.

Although Moeschlin agreed with Downey and Stasney[14] that the majority of young cells in the nodes of infectious mononucleosis should not be called lymphoblasts, he found lymphoblasts in normal and in other nonleukemic nodes. It is difficult to determine just what type of cell his lymphoblast (Fig. 7, *d*[23]) actually is. His figure showed a cell approximately 17 micra in diameter with a narrow rim of cytoplasm and prominent nucleoli, but with relatively coarse chromatin. Although no cells identical with Moeschlin's lymphoblast were seen in the nonleukemic nodes studied, cells of about the same size, with relatively coarse chromatin and with nucleoli, were occasionally seen in the poorly preserved parts of the imprints. In these same areas, it was often possible to see nucleoli in lymphocytes of all sizes. Because the morphologic picture seemed to be one due to damage and distortion, cells of this type were not included in the present study.

Although literature dealing with lymphocytogenesis in human nonleukemic nodes is sparse, there is a voluminous literature (the product largely of studies of animal or leukemic material) in which numerous authors express their views of the fundamentals of hematopoiesis. Downey discussed the early literature in 1927[8] and in 1938.[11] In a more recent publication,[12] it was again assumed that lymphocytogenesis in human nonleukemic nodes might closely parallel that seen in the nodes of rabbits and guinea pigs, and the discussion included in that report considered the various theories proposed for regeneration of lymphocytes. Since the results of the present investigation support the conclusions drawn in that study, no similar discussion will be included here.

A few points, however, require further clarification. The introduction of new terms into a science already encumbered by terminology is not desirable. All precursors of lymphocytes obviously are lymphoblastic in function, but un-

fortunately the term lymphoblast has been used in the past and is used in present-day clinical hematology to identify the most prominent lymphoid cell found in the blood in acute and subacute lymphatic leukemias. Most hematologists recognize the marked similarity of lymphoblasts and myeloblasts. Since cells conforming in morphology to the common conception of the term lymphoblast were not found in the nonleukemic nodes, it seems unwise to employ the name lymphoblast in *any* discussion of normal lymphocytogenesis. In an earlier publication,[12] lymphocyte regeneration was reconstructed as proceeding from undifferentiated reticular cells to reticular lymphocytes either directly or through a blast stage called the hematopoietic reticular cell or hemohistioblast. Mitoses were found to be most numerous in cells which appeared to be either hematopoietic reticular cells or reticular lymphocytes. Numerous transitional stages occurred between these two cell types, and the transition to ordinary lymphocytes was also a gradual one.

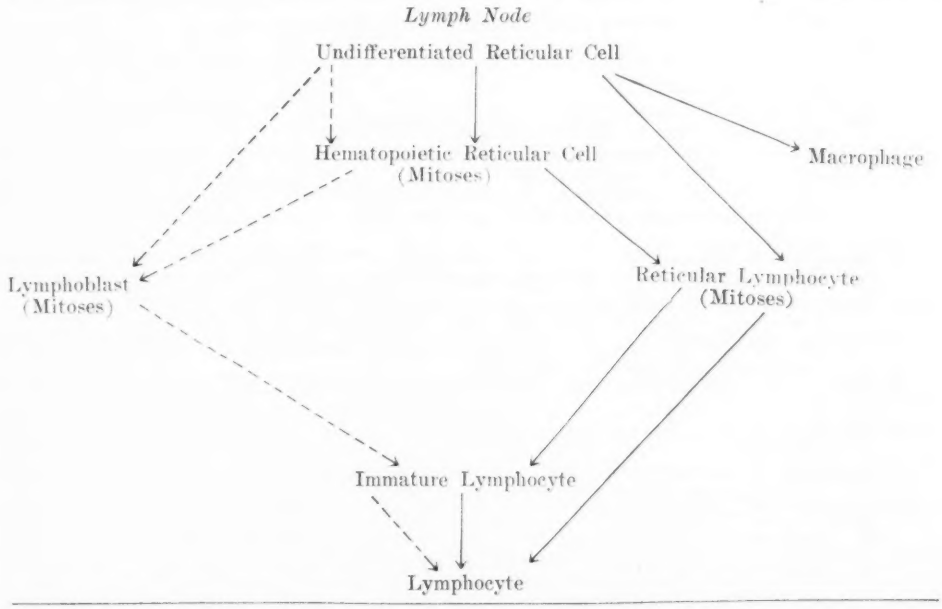
The problem is, for the most part, one of academic importance. However, in many instances an understanding of lymphocytogenesis is extremely valuable from a diagnostic standpoint. Various conditions are associated not only with peripheral lymphocytosis but also with the presence of a small percentage of immature lymphoid cells in the peripheral blood. These conditions are considered lymphatic reactions, reactions in which occasional normal immature lymphoid cells, reticular lymphocytes, enter the circulation. Reticular lymphocytes have been encountered in infectious mononucleosis, brucellosis, infectious hepatitis, some acquired hemolytic anemias, and in conditions in which there was pronounced eosinophilia. Because reticular lymphocytes may contain nucleoli and may exhibit a fine nuclear pattern (Figs. 12, 12′, 13), they are sometimes mistaken for lymphoblasts, and erroneous diagnoses of leukema are made. When it is understood that these reticular lymphocytes are merely immature lymphoid cells which may occur in any hyperplastic lymph node and which, when in the peripheral circulation, are usually simply the result of a benign left shift in lymphocytopoiesis, many of the problems of diagnosis will be simplified.

One noteworthy exception exists. In most chronic lymphatic leukemias, immature lymphocytes were not numerous in the peripheral blood. When immature cells were present, they were more similar to the reticular lymphocytes of infectious mononucleosis and of human nonleukemic nodes than to the lymphoblasts of acute leukemias. In fact, as has been stressed elsewhere,[22] there are cases of chronic lymphatic leukemia in which the peripheral blood so closely resembles that of some cases of infectious mononucleosis that a differential diagnosis on the basis of an examination of the blood smear is not always possible. The findings of reticular hyperplasia in node G is, therefore, not surprising; it serves merely to clarify previous impressions. Data available at the present time suggest that in chronic lymphatic leukemia, lymphocytogenesis closely parallels that seen in nonleukemic nodes. The clarity of morphologic detail in both mature and immature cells is, however, striking in many cases. It is possible that this clarity is the result of frequent mitoses with the production of enormous numbers of almost identical cells.

The presence of lymphoblasts in acute and subacute lymphatic leukemia and their absence in nonleukemic nodes is difficult to explain. Since they were not found in the present investigation, it is assumed that they do not occur in significant numbers in nonleukemic nodes. Transitional stages between the reticular lymphocytes of nonleukemic nodes and normal lymphocytes are often morphologically identical with transitional stages between lymphoblasts and normal appearing lymphocytes. Only the immature cells differ. That reticular cells may differentiate to lymphoblasts which undergo numerous mitoses and little if any differentiation is one explanation of the phenomenon.[15] It appears to be a very likely explanation, for in nodes, marrow, or blood from subacute and acute lymphatic leukemias, a few cells which show evidence of reticular origin can almost always be found.

Transitional stages encountered in this study can be organized into a diagramatic type of working hypothesis (Table II).

TABLE II. LYMPHOCYTOGENESIS



———, Transitional stages encountered in simple hyperplastic nodes, in nodes from infectious mononucleosis, and in nodes from chronic lymphatic leukemia.
- - - - - -, Transitional stages encountered in acute and subacute lymphatic leukemias.

SUMMARY

Relatively normal human lymph nodes, like the normal lymph nodes of rabbits and guinea pigs, do not contain cells morphologically identical with the myeloblasts of the bone marrow. The immature lymphoid cells (hematopoietic reticular cells and reticular lymphocytes) which are the progenitors of lymphocytes are morphologically distinguishable from myeloblasts. On this basis, it can be said that morphologic and functional dualism exists in normal adult

hematopoiesis; the hematopoietic reticular cell functions as a stem cell for lymphocytes, and the myeloblast functions as the stem cell for myeloid elements.

The results of the present investigation indicate that this condition prevails except in some cases of acute and subacute lymphatic leukemias where cells called lymphoblasts, many of which are morphologically indistinguishable from myeloblasts, occur in the blood, the lymph nodes, and the bone marrow.

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THE CLINICAL SIGNIFICANCE OF CELLULAR GIGANTISM IN HUMAN ERYTHROPOIESIS

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PLURINUCLEAR erythroblasts and pluripolar mitoses among erythroblasts in megaloblastic erythropoiesis have been described by a number of authors.[1, 7, 12-16, 19, 20] On the other hand, their occurrence in nonmegaloblastic erythropoiesis, which has been mentioned or illustrated by a few authors,[13-15, 17, 19, 20] is considered to be very unusual.[5, 8, 11, 15] In 1943 Limarzi and Levinson[15] reported a case in which they found what was considered to be a hitherto undescribed type of erythropoiesis in human sternal marrow. This was characterized by the formation of giant erythroblasts, giant plurinuclear erythroblasts having two or more nuclei, giant erythroblasts with lobulated nuclei, and abnormal mitoses with three or more poles. The erythroblasts appeared to be neither megaloblastic nor normoblastic; they were so abnormal and large that they were called gigantoblasts. The authors regarded the changes as irreversible and indicative of a cancerous process and proposed the terms erythroma or erythroblastoma. In 1944 Schleicher[19] reported two cases with similar atypical erythroblasts, chiefly of orthochromic type. He regarded them an expression of defunct reticulum, thereby implying that the changes were terminal or irreversible. Recently, Schwarz[20] reported a study of the question of cellular gigantism in human hematopoiesis based on observation of sternal marrow smears; he summarized the occurrence of giant plurinuclear erythroblasts and pluripolar mitoses among erythroblasts as phenomena independent of the type and condition of hematopoiesis. He concluded that gigantism does not depend on a morbid condition of erythropoiesis since it occurs in normal as well as in pathologic marrows. A somewhat contrary view regarding the significance of plurinuclear erythroblasts is expressed by Leitner[13] who states that atypical cell divisions are present mostly in serious disorders and are found in large numbers only in severe hemopathies.

In this laboratory we have observed a fairly regular appearance of plurinuclear erythroblasts in both normal and pathologic marrows, exclusive of the megaloblastic marrows in which it is generally agreed that these changes are common. In view of the alleged rarity of these conditions in nonmegaloblastic marrows (Limarzi and Levinson[15]) in contrast to its commonly observed occurrence in our experience and that of others (Schwarz[20] and Leitner[13]), and the divergence of opinion regarding their proper interpretation, it is desirable that material be reviewed for the purposes of shedding light on the following: (1) the incidence of the phenomena under question in normal and pathologic marrows, (2) the question of the reversibility of the changes, (3) the question of

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whether or not the changes are of agonal or terminal nature, (4) the question of whether or not erythropoiesis of the peculiar type here considered represents a new, different, or cancerous form of development, and (5) the presentation of data which may aid in the eventual interpretation of the clinical significance of such findings. For these objectives it is essential that cases for study are selected because of a significantly high incidence of plurinuclear erythroblasts, that more than a single marrow examination be available during life, and that none of the patients be in a terminal or agonal state. These criteria are fulfilled by the eight cases with pathologic marrows to be described in detail. In addition, control studies were carried out on eight normal individuals.

MATERIAL AND METHODS

The eight pathologic cases selected included one patient each with pernicious anemia, anemia associated with chronic blood loss, lymphoblastoma, monocytic leucemia, congenital hemolytic anemia before and after splenectomy, idiopathic thrombocytopenic purpura before and after splenectomy, cirrhosis of the liver, and acute hepatitis. The eight normal individuals selected for the control studies were healthy white males whose ages ranged from 18 to 53 years. They were chosen on the basis of their having had no serious illnesses and of having a sense of well-being, normal hematologic, chemical, and metabolic findings including the following: erythrocyte, leucocyte, platelet and reticulocyte counts, differential counts of leucocytes, bleeding and clotting times, capillary fragility, prothrombin and blood urea nitrogen levels, liver function tests, urinalyses, sedimentation rate, basal metabolic rate, electrocardiogram, stereoscopic chest x-ray, and thorough physical examination. In each case the sternal marrow was obtained, prepared, and examined by the methods previously described as routine for this laboratory.[2] Differential counts of the nucleated cells in the smears were based on examination of a minimum of 1,000 cells.

The age range of the control cases is not identical with that of the eight pathologic cases studied, but it is felt that they form a homogeneous age group with regard to sternal marrow findings, since Jacobsen's[9] study of bone marrows in seventy normal individuals ranging in age from 15 to 93 years reveals no significant variation due to differences of age. Furthermore, the fact that all the normal individuals in the control series are males does not detract from its reliability, since Segerdahl's[21] study of normal myelograms of 61 adult males and 49 adult females of comparable ages revealed only very slight variations in the myelograms due to difference of sex.

TERMINOLOGY

It has been pointed out that the phenomena of plurinuclearity and pluripolarity of mitosis are seen in both leucocytes and erythroblasts* of the sternal marrow.[13, 20] The present study is confined to the interpretation of these changes affecting erythroblasts. In the papers already cited, various terms have been used to denote the abnormal erythroblasts. Limarzi and Levinson refer to a very large uninuclear precursor of the abnormal cells and the large plurinuclear erythroblasts as giant erythroblasts and gigantoblasts after Ehrlich and Lazarus.[6] Schleicher refers to the large plurinuclear orthochromic cells as giant orthochromic erythroblasts. Schwarz described binucleated and plurinucleated erythroblasts and pluripolar mitoses. His descriptions and illustrations clearly indicate the identity of these forms with the plurinuclear cells and pluripolar mitoses described by the other authors. In our material, all types of plurinuclear erythroblasts as well as those with lobulated nuclei described by the other authors are represented, although, as will be shown, examples of such cells at the proerythroblast or orthochromic levels are very rare in normal marrow.

*The term erythroblast as used herein denotes any nucleated red cell which may undergo maturation changes from proerythroblast through basophilic, polychromatophilic, and orthochromic stages before final denucleation.

MORPHOLOGY OF PLURINUCLEAR ERYTHROBLASTS AND PLURIPOLAR MITOSES AMONG ERYTHROBLASTS

Limarzi and Levinson described three main types of abnormal erythropoiesis. In the first type, a giant uninuclear erythroblast gave origin to plurinuclear cells by a series of multiple and complicated mitotic divisions without cytoplasmic division. The second type was characterized by a process of folding, indentation, lobulation, and constriction of the original nucleus so that eventually several

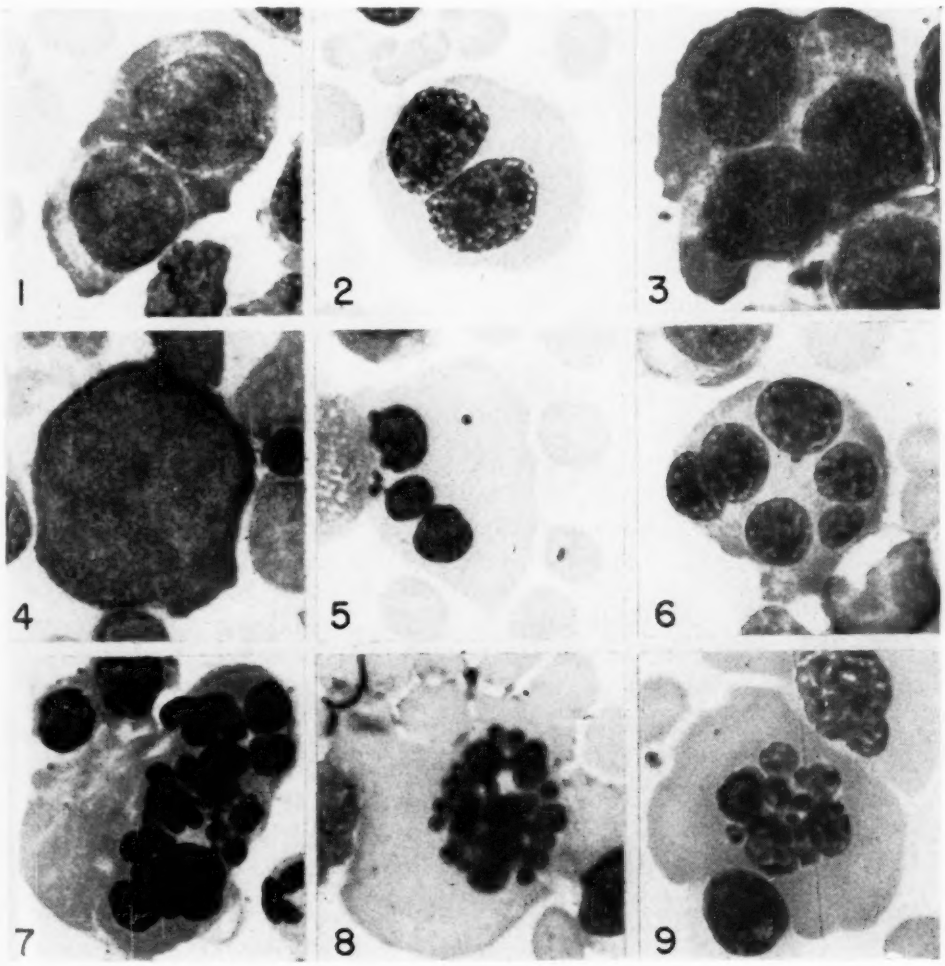

PLATE I

1.—Case 1. Binuclear proerythroblast 17 x 33 microns.
2.—Normal. Binuclear polychromatophilic erythroblast 21 x 29 microns.
3.—Case 1. Trinuclear early basophilic erythroblast 26 x 36 microns.
4.—Case 2. Trinuclear proerythroblast 25 x 27 microns.
5.—Case 1. Trinuclear orthochromic erythroblast 18 x 27 microns.
6.—Case 7. Sesquinuclear polychromatophilic erythroblast 21 x 26 microns.
7.—Case 2. Late polychromatophilic erythroblast with many nuclear masses 21 x 36 microns.
8.—Case 1. Orthochromic erythroblast with many nuclear masses (karyorrhexis ?) 25 x 37 microns.
9.—Case 1. Late polychromatophilic erythroblast with fused or lobulated nuclei 20 x 30 microns.

independent nuclei were produced in a single cell. The third type was characterized by complete or incomplete complicated amitosis without cytoplasmic division. In Schwarz's material, examples of all these forms are described and illustrated. The giant orthochromic erythroblasts in Schleicher's material had diameters ranging from 15.8 to 39.6 microns. The nuclei were either lobulated, fragmented, or consisted of from five to eight deeply staining masses of irregular size.

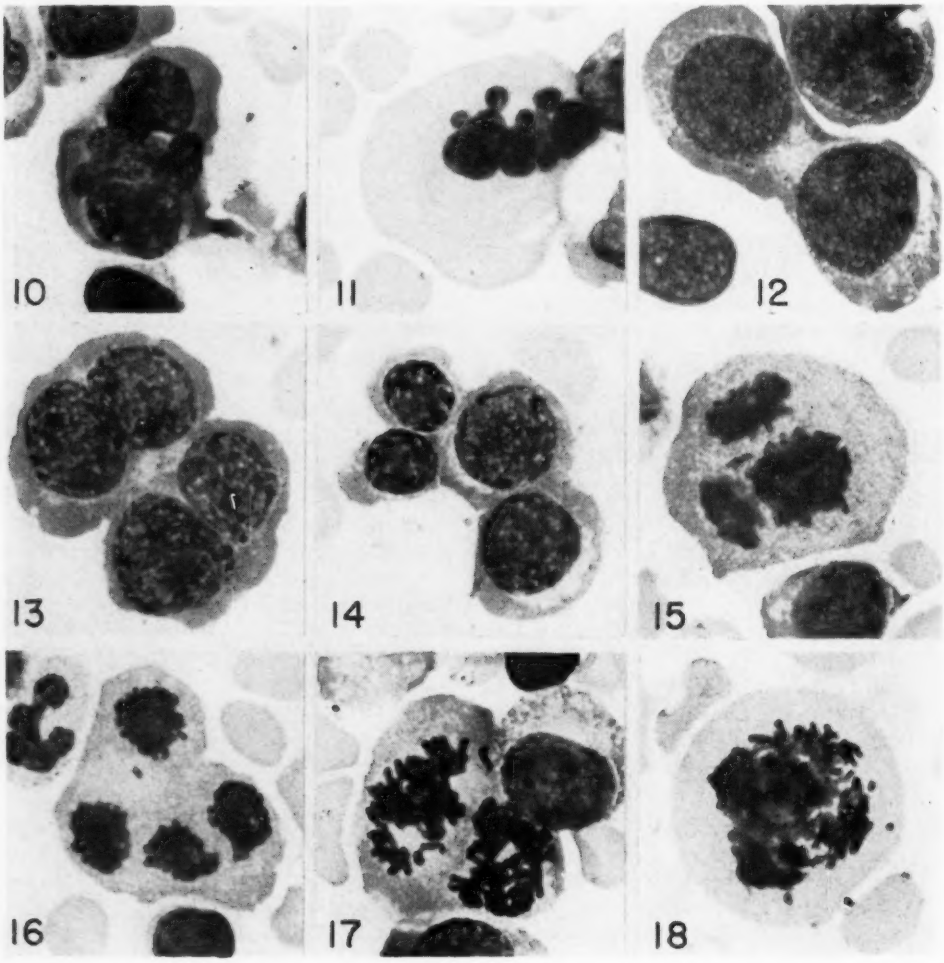

PLATE II

10.—Case 1. Basophilic erythroblast with lobulated nucleus 17 x 28 microns.
11.—Case 1. Orthochromic erythroblast with lobulated nuclei and accessory nuclear masses (karyorrhexis ?) 21 x 27 microns.
12.—Case 1. Incomplete cytokinesis in binuclear proerythroblast 17 x 40 microns.
13.—Case 2. Incomplete cytokinesis in quadrinuclear early basophilic erythroblast 25 x 37 microns.
14.—Case 2. Incomplete cytokinesis in two binuclear basophilic erythroblasts.
15.—Case 1. Tripolar mitosis with aberrant chromosomal mass in basophilic erythroblast 23 x 27 microns.
16.—Case 1. Quadripolar mitosis with aberrant chromosomal mass in polychromatophilic erythroblast 19 x 30 microns.
17.—Case 1. Irregular pluripolar mitosis in polychromatophilic erthroblast 20 x 27 microns.
18.—Case 1. Pluripolar mitosis with at least 7 asters in late polychromatophilic erythroblast 26 x 28 microns.

The published detailed descriptions and illustrations of plurinuclear cells and pluripolar mitoses[15, 17, 19, 20] in both megaloblastic and nonmegaloblastic marrows are very complete and require little elaboration. In Plates I and II* are illustrated examples of such cells from one normal and three pathologic cases in our material. Examples from a case of pernicious anemia are omitted because of the general agreement as to their common occurrence in that disease. The identity of the cells illustrated and those represented by others is clear. It is of special interest that the condition of plurinuclearity in our material did not interfere with the relative progress of nuclear and cytoplasmic maturation. With very few exceptions, plurinuclear cells with basophilic erythroblastic nuclei were found to have cytoplasm characteristic of that maturation level. Similar normal relationships were observed in the polychromatophilic and orthochromic levels. These findings are in agreement with those of Schwarz whose study indicates that plurinuclearity does not interfere with the development and functional activities of the erythroblasts. Furthermore, the type of maturation and the morphologic forms of both nuclei and cytoplasm in the plurinuclear cells, in every instance, conformed to that of the prevailing type of erythopoiesis. In the normoblastic marrows (Cases 2, 4, 5, 6, 8, in addition to all normal cases) the plurinuclear cells had nuclear and cytoplasmic characteristics entirely similar to those of the remaining uninuclear normoblasts; in the megaloblastic marrow (Case 3), not only did the nuclear and cytoplasmic characteristics of the plurinuclear cells conform to that of the megaloblasts which predominated, but the relative rates of maturation of nuclei and hemoglobinization of cytoplasm were characteristic of the cells of the megaloblastic line, as described elsewhere.[3] In two instances (Cases 1 and 7) erythropoiesis involving the uninuclear erythroblasts could not be identified as either normoblastic or megaloblastic. The erythroblasts were classified as dysplastic, being somewhat intermediate in morphology between normoblasts and megaloblasts. In these marrows the plurinuclear erythroblasts and the remaining uninuclear erythroblasts had similar morphologic features. The sizes of the giants were approximate multiples of those of the corresponding stages of the uninuclear cells, roughly directly proportional to the amount of nuclear material, that is, the number of nuclei in each cell.

INCIDENCE OF PLURINUCLEAR ERYTHROBLASTS IN NORMAL HUMAN STERNAL MARROW

The literature does not contain statistical data concerning the incidence of plurinuclear erythroblasts or pluripolar mitoses among erythroblasts in normal human sternal marrow. Limarzi and Levinson stated that the occurrence of normal bipolar and pathologic multipolar mitosis of erythroid cells in the same marrow is an unusual finding in the adult. Schwarz stated that gigantism occurs in normal as well as in pathologic marrows, and also that he repeatedly encountered pluripolar mitoses in normoblastic regeneration, even in normal marrows. The myeloid:erythroid ratios and incidences of plurinuclear erythroblasts in normal individuals are shown in Table I.

*The photomicrographs were prepared by Frank N. Ruslander.

TABLE I. MYELOID:ERYTHROID RATIOS* AND INCIDENCE OF PLURINUCLEAR ERYTHROBLASTS IN STERNAL MARROW OF NORMAL INDIVIDUALS

CASE	AGE	M:E RATIO	PNE(‰)	TOTAL ERB
1	18	3:1	5.0	13,065
2	22	2:1	2.0	5,010
3	24	2:1	2.2	5,011
4	30	2:1	1.2	5,006
5	31	2:1	1.0	5,005
6	36	2:1	2.8	5,014
7	45	2:1	1.0	5,005
8	53	2:1	5.1	10,051

M:E ratio, Myeloid: erythroid ratio.

PNE (‰), number of plurinuclear erythroblasts per 1,000 uninuclear erythroblasts.

Total ERB, number of erythroblasts examined.

*The myeloid: erythroid ratio represents the ratio of the number of leuocytes of myeloid origin, including monocytes, to the number of erythroblasts in the smears. It must be pointed out that the value is relative, not absolute. A decreased M:E ratio is indicative of a relative increase of erythropoiesis or relative decrease of myeloid leuopoiesis. The ratio cannot be used to determine absolute increase or decrease of erythropoiesis. However, our practice includes the preparation of gross marrow units which are evaluated by the method suggested by Schleicher.[13] In the presence of a cellular marrow with increased or normal frequency of erythroblastic islands, as determined by examination of the histologic pattern, a significantly lowered M:E ratio may be taken as a rough measure of the degree of acceleration or increase of erythropoiesis.

In the control series the incidences of plurinuclear erythroblasts vary from 1.0 to 5.1 per 1,000 uninuclear erythroblasts. The myeloid:erythroid ratios vary from 2:1 to 3:1. On the basis of the normal material available at present, it must be concluded that a myeloid:erythroid ratio significantly lower than 2:1 and an incidence of plurinuclear erythroblasts significantly greater than 5.1 per 1,000 uninuclear erythroblasts are to be regarded as abnormal. Furthermore, the presence of an appreciable number of pluripolar mitoses is abnormal, as indicated by the fact that in a total of 53,167 erythroblasts in eight normal persons, not a single example of pluripolar mitosis was seen. In addition, no examples of high plurinuclearity, that is, cells with more than two nuclei were encountered in normal marrows. Finally, the differential distribution of the plurinuclear erythroblasts, represented in Table II, reveals the relative rarity of plurinuclear pronormoblasts and plurinuclear orthochromic normoblasts. It is noteworthy that the differential distribution of the plurinuclear cells is similar to that of the normoblasts in general in normal individuals.

TABLE II. DIFFERENTIAL DISTRIBUTION OF PLURINUCLEAR AND UNICLEAR NORMOBLASTS IN NORMAL HUMAN STERNAL MARROW

	PNE	UNE
Total cells examined	167	2000
Pronormoblasts	1%	2%
Basophilic normoblasts	24%	11%
Polychromatophilic normoblasts	74%	86%
Orthochromic normoblasts	1%	1%

PNE, plurinuclear erythroblasts.

UNE, uninuclear erythroblasts.

One must conclude that the alleged rarity of pluripolar mitoses among erythroblasts in normal sternal marrow is an actuality, whereas the alleged rarity of plurinuclear erythroblasts must be explained on the basis of their having been overlooked in routine examination of marrow smears, due to their relatively small incidence.

INCIDENCE OF PLURINUCLEAR ERYTHROBLASTS AND PLURIPOLAR MITOSES AMONG ERYTHROBLASTS IN PATHOLOGIC HUMAN STERNAL MARROWS

Limarzi and Levinson present data indicating the incidence of cells classified as atypical erythroid cells, which we assume to represent the giant erythroblasts in their material, as 63.8 per 1,000 typical erythroblasts.* Only one study was available during life. Schwarz does not offer statistical data regarding the incidence of plurinuclear cells. It is stated, however, that one case of myeloblastic leucemia was "quite outstanding in the degree and the frequency of the abnormality in question."[20] Schleicher gave the ratio of giant orthochromic erythroblasts to late pronormoblasts as 4 to 1 in one case, and he recorded the ratio of giant orthochromic erythroblasts to early and late basophilic erythroid cells as 3 to 1 in his second case. He did not discuss the significance of these ratios.

The incidences of plurinuclear erythroblasts in our pathologic marrows are shown in Table III. They vary from 16.0 to 159.6 per 1,000 uninuclear erythroblasts in the initial studies. Obviously, these data do not reflect the actual incidence of plurinuclear cells in pathologic marrows, since no attempt was made to survey all of our material. It can be stated, however, that rapid scanning of several slides with the low-power objective suffices for the discovery of such cells in bone marrow concentrate smears in which their frequency is in the neighborhood of 30 per thousand erythroblasts. They are easily noticed because of their large size. Plurinuclear erythroblasts easily may be overlooked in normal marrow smears because of their infrequent occurrence. On the other hand, it is difficult to understand how plurinuclear erythroblasts can be overlooked in pathologic marrows, especially in instances of increased erythropoiesis, since they are frequently present in our material and that of others.[13, 20] In Cases 1, 2, and 3 with incidences of 159.6, 99.2, and 52.8, respectively, the cells were so numerous that the smears presented a striking pathologic picture on

TABLE III. INCIDENCES OF PLURINUCLEAR ERYTHROBLASTS, MYELOID:ERYTHROID RATIOS, AND ERYTHROCYTE COUNTS IN PATHOLOGIC HUMAN STERNAL MARROWS

CASE	DIAGNOSIS		PNE(‰)	M:E RATIO	RBC
1	Lymphoblastoma (?)	First study	159.6	1:1	1.84
		Second study	13.8	1:1	2.34
2	Chronic hemorrhage	First study	99.2	1:1	1.30
		Second study	5.4	1:2	1.40
3	Pernicious anemia	First study	52.8	1:2	0.73
		Second study	4.4	2:1	3.63
4	Hepatitis	First study	35.8	1:1	3.17
		Second study	6.0	3:1	4.00
5	Congenital hemolytic anemia	First study	32.0	1:2	1.13
		Second study	5.2	7:1	4.33
6	Cirrhosis of liver	First study	23.0	1:1	2.52
		Second study	6.0	7:1	3.80
7	Monocytic leucemia	First study	18.8	2:1	2.00
		Second study	22.0	2:1	2.21
8	Thrombocytopenic purpura	First study	16.0	1:2	2.26
		Second study	3.8	2:1	4.42

PNE(‰), number of plurinuclear erythroblasts per 1,000 uninuclear erythroblasts.
M:E ratio, myeloid:erythroid ratio.
RBC, erythrocyte count in millions per cubic millimeter of blood.

*Calculated from their data.

first inspection. The pathologic marrows also afforded excellent opportunities for examination of pluripolar mitoses (see individual case reports).

ON THE QUESTION OF THE TERMINAL NATURE OF THE PHENOMENON OF PLURINUCLEAR OF ERYTHROBLASTS

Schleicher's observation of two cases with giant orthochromic erythroblasts in patients with terminal illnesses, and Limarzi and Levinson's single case with terminal disease cannot be accepted as evidence of the terminal nature of the findings, since our series includes several cases showing an abnormal incidence of plurinuclear erythroblasts with clinical recovery (Table III). It is essential that serial marrow studies be made on patients exhibiting the change so that remission from the underlying clinical disease with a concomitant disappearance of the condition of plurinuclearity may not be overlooked.

ON THE QUESTION OF IRREVERSIBILITY AND CANCEROUS NATURE OF THE CHANGES

Some authors[15, 19] have considered the phenomenon under discussion to represent an irreversible change in the marrow or an indication of a defunct reticulum. As has been pointed out, the marrow pattern, in so far as erythropoiesis is concerned, may revert to normal after exhibiting a temporary period during which plurinuclear cells may be formed. The data already presented are evidence in favor of the concept of the reversibility of the lesion. The belief that the lesion is of cancerous nature[15] is partly based on the assumption that the changes are irreversible. Furthermore, it has been stated that bipolar mitosis and multipolar mitosis of erythroid cells in the same marrow is an unusual finding in the adult.[15] Our data do not conform to either of these assumptions. According to Schwarz, pluripolar mitosis does not represent a more fertile type of regeneration, as suggested by Limarzi, Jones, and Levinson,[14] nor is his material in accord with a biologic transformation akin to neoplastic deviation as assumed by these authors.

Although Schwarz does not present serial marrow studies on his cases showing plurinuclear erythroblasts, he concludes, largely on the basis of experimental evidence, that once normal conditions are restored the plurinuclear erythroblast tends to divide into as many daughter cells as there were nuclei formed by the preceding karyokinesis. The experimental work of Chambers,[4] cited by Schwarz as evidence for the reversibility of the phenomenon of suppressed cytokinesis, is based on the observations of sea-urchin eggs subjected to hypertonic sea water and later replaced in sea water, late amphiaster eggs placed in 2½ per cent ether solution in sea water and replaced within five minutes in sea water, late amphiaster eggs subjected to contact with 1½ to 2 per cent ether solutions in sea water or hypotonic solutions, and amphiaster eggs subjected to mechanical agitation. The following case reports are presented as evidence of reversibility of the changes under question in patients with clinical disease.

CASE REPORTS

CASE 1.—A white woman, aged 65 years, stated that she felt well until December, 1943, when she began to experience weakness, malaise, dyspnea, and weight loss. The only sig-

nificant findings on physical examination were enlarged liver and spleen. The edge of the liver was 5 cm. below the costal margin and the inferior border of the spleen extended below the umbilicus. On March 8, 1946, the initial blood studies showed: erythrocytes, 1.84 millions per cubic millimeter; hemoglobin, 7.7 grams per cent; reticulocytes, 1.5 per cent; leucocytes, 1,900 per cubic millimeter; platelets, 61,000 per cubic millimeter; mean corpuscular volume, 109 cubic microns; mean corpuscular hemoglobin, 42 micromicrograms. The differential distribution of the leucocytes was: band form neutrophiles, 19 per cent; polymorphonuclear neutrophiles, 21 per cent; monocytes, 23 per cent; lymphocytes, 38 per cent.

The bone marrow study revealed hypercellular marrow with marked increase in the frequency of erythroblastic islands as observed by inspection of gross marrow units (Schleicher[18]). The myeloid:erythroid ratio was 1:1. Erythropoiesis was atypical, characterized by the presence of dysplastic erythroblasts having a superficial resemblance to megaloblasts because of their large size and relatively vesicular chromatin networks in the nuclei. There were also numerous plurinuclear erythroblasts which were present in a frequency of 159.6 per thousand uninuclear erythroblasts (Table III). Myeloid leucopoiesis was relatively reduced, with band form neutrophiles predominating. Megakaryocytogenesis was reduced. There were 49 per cent lymphocytes. Numerous monocytoid cells recently derived from reticulum appeared in isolated instances and in syncytial masses with two to four nuclei. These resembled cells the author had previously encountered in imprints of lymph nodes from cases of reticulum cell type of lymphoblastoma, and they recall Schleicher's description of monocytoid reticulum cells in his Case 1, proved at autopsy to be reticulum cell sarcoma. The blood and marrow findings were summarized as macrocytic hyperchromic anemia, neutropenia, thrombocytopenia, probable reticulum cell type of lymphoblastoma with involvement of bone marrow, liver, and spleen, and accelerated dysplastic erythropoiesis with increased frequency of plurinuclear erythroblasts. In addition, there were numerous examples of pluripolar mitoses among erythroblasts (Plates I and II). Their incidence was 9.6 per thousand erythroblasts.

The patient received transfusions, vitamin B_6, and oral folic acid. After twenty days, the appetite had returned and she began to gain weight and strength rapidly. On May 12, 1946, the blood study showed: erythrocytes, 2.11 millions per cubic millimeter; hemoglobin, 5.5 grams per cent; reticulocytes, 15.8 per cent; leucocytes, 3,000 per cubic millimeter; mean corpuscular volume, 107 cubic microns; mean corpuscular hemoglobin, 26 micromicrograms.

The bone marrow study revealed hypercellular marrow, as previously. The myeloid: erythroid ratio was 1:1. The most significant change from the previous myelogram was a reduction in the incidence of plurinuclear erythroblasts from 159.6 to 13.8 per 1,000 uninuclear erythroblasts (Table III). There were only 2.4 per 1,000 pluripolar mitoses in the second study.

Comment.—The case demonstrates the variability of the frequency of plurinuclear erythroblasts and pluripolar mitoses in a single patient. It is noteworthy that the reduction in their incidences occurred with improvement of the clinical condition of the patient and a reduction of the severity of the anemia. To be sure, the second study revealed that the changes were still present in a degree significantly greater than normal; but in view of the probability that the patient has a neoplastic disease affecting the liver, spleen, and bone marrow, the failure of the abnormality of erythropoiesis to disappear completely is to be expected. Furthermore, erythropoiesis was still accelerated at the second examination.

CASE 2.—A 52-year-old white man was admitted to the hospital June 3, 1946, with a history of dyspnea, weakness, tarry stools, and occasional vomiting. Three weeks before admission he drank an unknown quantity (approximately 2 pints) of a commercial shellac solvent. The significant findings were marked pallor, firm and barely palpable liver, and severe thrombosed and bleeding hemorrhoids. The serologic tests for syphilis were positive.

The stools revealed traces to 4 plus occult blood during the entire hospital stay. Repeated studies for ova and parasites gave negative results. Two x-ray examinations of the entire gastrointestinal tract failed to reveal any sources of the blood in the stools. The only known sources were hemorrhoids, which were very prominent. The patient refused surgical treatment. Biopsy of the liver showed a normal histologic structure. The initial blood study showed: erythrocytes, 1.30 millions per cubic millimeter; hemoglobin, 2.9 grams per cent; reticulocytes, 7.2 per cent; leucocytes, 6,500 per cubic millimeter; platelets, 100,000 per cubic millimeter; mean corpuscular volume, 77 cubic microns; mean corpuscular hemoglobin, 22 micromicrograms.

The bone marrow study revealed hypercellular marrow with marked increase of erythropoiesis and a myeloid:erythroid ratio of 1:1. Erythropoiesis was normoblastic. There were 99.2 plurinuclear erythroblasts and 3.1 pluripolar mitoses per 1,000 uninuclear normoblasts (Table III and Plates I and II). Myeloid leucopoiesis and megakaryocytogenesis were unaltered. There were 13 per cent lymphocytes. The blood and marrow findings were summarized as microcytic hypochromic anemia, neutropenia, thrombocytopenia, and accelerated normoblastic erythropoiesis with increased frequency of plurinuclear erythroblasts.

During a period of observation from June 6 to 26, 1946, iron therapy and transfusions were withheld. On June 26 the blood study showed: erythrocytes, 1.40 millions per cubic millimeter.

The bone marrow findings were similar to those of the first study, with the exception that the incidence of plurinuclear cells had dropped from 99.2 to within a normal level of 5.4 per 1,000 uninuclear erythroblasts (Table III). No pluripolar mitoses were seen among 5,000 erythroblasts.

Comment.—The case illustrates a remission from the condition of atypical plurinuclear erythropoiesis, in spite of the continued presence of severe anemia and accelerated erythropoiesis.

CASE 3.—A 62-year-old white man with idiopathic pernicious anemia experienced an hematologic remission as a result of folic acid therapy. The initial blood study showed: erythrocytes, 730,000 per cubic millimeter; hemoglobin, 2.7 grams per cent; reticulocytes, 1.7 per cent; leucocytes, 5,800 per cubic millimeter; platelets, 16,000 per cubic millimeter; mean corpuscular volume, 122 cubic microns; mean corpuscular hemoglobin, 38 micromicrograms.

The bone marrow study revealed hypercellular marrow with increased erythropoiesis of megaloblastic type and a myeloid:erythroid ratio of 1:2. There were 52.8 plurinuclear erythroblasts and 2.8 pluripolar mitoses per 1,000 uninuclear erythroblasts (Table III). Only an occasional plurinuclear erythroblast had normoblastic character; the remaining plurinuclear erythroblasts were megaloglastic. Myeloid leucopoiesis revealed changes characteristic of antipernicious anemia principle deficiency.[10] Megakaryocytogenesis was reduced.

At the time of the second marrow study, the blood showed: erythrocytes, 3.63 millions per cubic millimeter; hemoglobin, 10.7 grams per cent. The second bone marrow study revealed cellular marrow with slightly increased erythropoiesis and a myeloid:erythroid ratio within the normal range (2:1). Erythropoiesis was entirely normoblastic. The incidence of plurinuclear erythroblasts had dropped from 52.8 to 4.4 per 1,000 uninuclear erythroblasts (Table III). Only one pluripolar mitosis was found among 10,000 erythroblasts.

Comment.—The case illustrates the complete remission of the condition of excessive plurinuclearity and pluripolarity among erythroblasts in megaloblastic marrow as a result of specific therapy.

CASE 4.—A 54-year-old white man was admitted because of mild acute hepatitis. Serologic tests for leptospira were negative. Various liver function tests revealed only slight disturbances. The initial blood study on Oct. 16, 1946, showed: erythrocytes, 3.17 millions per cubic millimeter; hemoglobin, 11.6 grams per cent; reticulocytes, 6.6 per cent; leucocytes, 5,500 per cubic millimeter; platelets, 300,000 per cubic millimeter; mean corpuscular volume, 100 cubic microns; mean corpuscular hemoglobin, 37 micromicrograms.

The bone marrow study revealed slightly hypercellular marrow with increased erythropoiesis and a myeloid:erythroid ratio of 1:1. Erythropoiesis was normoblastic. There were 35.8 plurinuclear erythroblasts and 1.0 pluripolar mitoses per 1,000 uninuclear erythroblasts (Table III). The blood and marrow findings were summarized as macrocytic normochromic anemia and accelerated normoblastic erythropoiesis with increased frequency of plurinuclear erythroblasts.

The icterus cleared and the patient remained asymptomatic. A final blood study on Nov. 12, 1946, showed: erythrocytes, 4.00 millions per cubic millimeter; hemoglobin, 13.3 grams per cent; leucocytes, 7,150 per cubic millimeter.

The second bone marrow study revealed approximately normal cellularity and a myeloid:erythroid ratio of 3:1. The only significant finding in the myelogram was evidence of a decrease of the incidence of plurinuclear erythroblasts from 35.8 to 6.0 per 1,000 uninuclear cells (Table III). One pluripolar mitosis was seen.

Comment.—The case illustrates that the temporary condition of excessive plurinuclearity of erythroblasts may occur in relatively mild clinical disease.

CASE 5.—A white woman, aged 18 years, with congenital hemolytic anemia, was admitted because of an acute hemolytic episode. The initial blood study on Nov. 3, 1943, showed: erythrocytes, 1.13 millions per cubic millimeter; hemoglobin, 4.6 grams per cent; reticulocytes, 13 per cent; leucocytes, 26,400; platelets, 400,000 per cubic millimeter; mean corpuscular volume, 92 cubic microns; mean corpuscular hemoglobin, 41 micromicrograms. There were 60 normoblasts per 100 leucocytes. The erythrocytes were deeply staining cells of small diameter (spherocytes).

The bone marrow study revealed hyperplastic marrow with a myeloid:erythroid ratio of 1:2. Erythropoiesis was normoblastic. There were 32.0 plurinuclear erythroblasts and 1.8 pluripolar mitoses per 1,000 uninuclear erythroblasts (Table III). The blood and marrow findings were summarized as normocytic normochromic anemia with spherocytosis, normoblastic crisis, neutrophilic leucocytosis, and accelerated normoblastic erythropoiesis with increased frequency of plurinuclear erythroblasts. Splenectomy was performed Nov. 11, 1943, without complications.

At the time of the second marrow study on Nov. 23, 1943, the blood showed: erythrocytes, 4.33 millions per cubic millimeter; hemoglobin, 12.4 grams per cent. The second marrow study revealed hypercellular marrow with a myeloid:erythroid ratio of 7:1, revealing that the chief cause of the hypercellularity was increased leucopoiesis. The incidence of plurinuclear erythroblasts had fallen from 32.0 to 5.2 per 1,000 uninuclear erythroblasts (Table III), and only one pluripolar mitosis was seen among 7,000 erythroblasts.

Comment.—The case illustrates the remission of the condition of excessive plurinuclearity after removal of an important factor (hypersplenism) in the pathogenesis of the clinical disease.

CASE 6.—A 32-year-old white woman was admitted with cirrhosis of the liver, proved by biopsy. The initial blood study on June 16, 1946, showed: erythrocytes, 2.52 millions per cubic millimeter; hemoglobin, 9.4 grams per cent; reticulocytes, 2.4 per cent; leucocytes, 10,550 per cubic millimeter; platelets, 126,000 per cubic millimeter; mean corpuscular volume, 135 cubic microns; mean corpuscular hemoglobin, 37 micromicrograms.

The bone marrow study revealed hyperplastic marrow with a myeloid:erythroid ratio of 1:1. Erythropoiesis was normoblastic, with an incidence of 23.0 plurinuclear erythroblasts and 2.8 pluripolar mitoses per 1,000 uninuclear erythroblasts (Table III). The blood and marrow studies were summarized as macrocytic normochromic anemia, thrombocytopenia, and accelerated normoblastic erythropoiesis with increased frequency of plurinuclear erythroblasts.

The patient received various forms of therapy, following which her clinical condition improved. Four days before her discharge the blood study showed: erythrocytes, 3.88 millions per cubic millimeter; hemoglobin, 9.2 grams per cent.

The second marrow study revealed slightly hypercellular marrow without increased incidence of plurinuclear erythroblasts, which were now 6.0 per 1,000 uninuclear erythroblasts. There was only one pluripolar mitosis in approximately 12,500 erythroblasts.

Comment.—The case illustrates the disappearance of excessive plurinuclearity in a patient with a permanent lesion such as cirrhosis of the liver, concomitant with clinical improvement.

CASE 7.—A 27-year-old white man was admitted with monocytic leucemia. The initial blood studies showed: erythrocytes, 2.00 millions per cubic millimeter; hemoglobin, 4.5 grams per cent; reticulocytes, 0.1 per cent; leucocytes, 15,000; platelets, 30,000 per cubic millimeter; mean corpuscular volume, 75 cubic microns; mean corpuscular hemoglobin, 22.5 micromicrograms. The differential distribution of the leucocytes was: myeloblasts, 2 per cent; promyelocytes, 1 per cent; myelocytes, 1 per cent; metamyelocytes, 3 per cent; band form neutrophiles 26 per cent; polymorphonuclear neutrophiles, 14 per cent; eosinophile promyelocytes, 1 per cent; eosinophiles, ½ per cent; monocytes and immature monocytoid cells, 26 per cent; lymphocytes, 25 per cent; plasma cells, ½ per cent; megakaryocytic nuclei, 1 per cent; atypical polychromatophilic erythroblasts, 3 per 100 leucocytes. There were numerous giant platelets.

The bone marrow study revealed hyperplastic marrow with a myeloid:erythroid ratio of 2:1. Erythropoiesis was of the dysplastic type, resembling that seen in Case 1. There were 18.8 plurinuclear erythroblasts, and an occasional pluripolar mitosis per 1,000 uninuclear erythroblasts (Table III). Myeloid leucopoiesis was altered because of the development of monocytoid stem cells and monocytes, which comprised a total of 25 per cent of the nucleated cells. The hematologic findings were diagnostic of monocytic leucemia, Naegeli type. The patient received various forms of therapy, without effect.

Five months later, the marrow study revealed findings similar to those seen in the initial examination, with the exception that atypical stem cells and monocytoid stem cells were more numerous and that the incidence of plurinuclear erythroblasts had increased slightly from 18.8 to 22.0 per 1,000 uninuclear erythroblasts.

Comment.—This case is included by way of contrast with the others, since it illustrates the persistence of the plurinuclear trait in a patient whose clinical condition became worse during the period of observation.

CASE 8.—A 23-year-old white woman with idiopathic thrombocytopenic purpura was admitted to the hospital because of approximately five months of purpura and bleeding from mouth and vagina. The initial blood study showed: erythrocytes, 2.26 millions per cubic millimeter; hemoglobin, 6.7 grams per cent; reticulocytes, 24.5 per cent; leucocytes, 12,400 per cubic millimeter; platelets, 1,900 per cubic millimeter; mean corpuscular volume, 93 cubic microns; mean corpuscular hemoglobin, 29.6 micromicrograms. There were 4 normoblasts per 100 leucocytes.

The bone marrow study revealed hyperplastic marrow with a myeloid:erythroid ratio of 1:2. Erythrocytogenesis was normoblastic, with 16.0 plurinuclear erythroblasts and 2.2 pluripolar mitoses per 1,000 uninuclear erythroblasts (Table III). Megakaryocytogenesis was normal quantitatively. Splenectomy was performed without complications on Oct. 4, 1946.

All hemorrhagic and purpuric tendencies had disappeared by the fourth postoperative day. On Nov. 11, 1946, the blood showed: erythrocytes, 4.42 millions per cubic millimeter; platelets, 90,000 per cubic millimeter.

The second marrow study revealed approximately normal cellularity, with a myeloid: erythroid ratio of 2:1. The only other significant change in the myelogram was the appearance of evidence that the incidence of plurinuclear erythroblasts had dropped from 16.0 to 3.8 per 1,000 uninuclear erythroblasts. One pluripolar mitosis was seen among 5,000 erythroblasts.

Comment.—The case is an additional example of the disappearance of the condition of excessive plurinuclearity of erythroblasts concomitant with clinical and hematologic improvement following the removal of one of the important factors (hypersplenism) in the pathogenesis of the associated clinical disease.

DISCUSSION

The case reports presented include examples illustrating the temporary or reversible nature of the condition of plurinuclearity of erythroblasts and of pluripolarity of mitoses among erythroblasts in patients with either mild or serious clinical diseases (Cases 2, 3, 4, 5, 6, 8). The importance of such findings is obvious when applied to the concept of a cancerous nature of the changes. The fact that plurinuclear erythroblasts are present in normal marrows is additional evidence against their cancerous nature. Furthermore, the differential distributions and morphologic characters of the plurinuclear cells are remarkably similar to those of the uninuclear cells in the pathologic as well as the normal cases. This is suggestive of the action of the etiologic factors on any types of erythroblasts which are present at the time such factors come into play. Thus, the plurinuclear forms under question may be regarded as variants of the prevailing types of erythroblasts and not as members of a new genetic line of cells. In Cases 1 and 7 (lymphoblastoma and leucemia) with dysplastic erythropoiesis, the atypical giant plurinuclear cells could easily have been interpreted as representatives of a new cell line. However, the morphologic forms of the plurinuclear cells appeared to be modifications of the prevailing erythroblasts, readily explained by suppression of cytokinesis alone. It cannot be argued that because two of our cases occurred in association with malignant disease (Case 1, lymphoblastoma, and Case 2, leucemia), plurinuclearity of erythroblasts is related to malignant proliferation of cells in the bone marrow, since none of the other cases had any relationship to malignant disease. We have recently studied a case of polycythemia vera of several years' duration, now terminating as myelogenous leucemia. The bone marrow in this patient reveals only a normal incidence of plurinuclear cells.

A patient with chronic microcytic hypochromic anemia (Case 2) revealed a marked decrease in the incidence of plurinuclear erythroblasts to normal levels in spite of absence of clinical or hematologic improvement. While in most cases excessive plurinuclearity among erythroblasts was associated with accelerated erythropoiesis, it may be absent, or it may disappear, as in Case 2 in which a possible toxic agent (ingested shellac solvent) may have played an etiologic role.

The occurrence of the phenomena in relation to a progressive unfavorable course in patients who ultimately succumb to their clinical disease is not acceptable evidence in favor of an irreversible change. Indeed, the apparent nonreversibility is the result, not the cause, of the underlying clinical condition. Additional data may determine a range of incidences of plurinuclearity which is indicative of nonreversibility, but the present material shows that incidences of 99.2 and 159.6 per 1,000 are below such limits.

The present study does not disclose the action of factors common to all the cases. It is possible that when more cases illustrating plurinuclearity of high incidence are accumulated, such factors may be discovered. At any rate, the changes we have described, which involve suppression of cytokinesis without interference with maturation or karyokinesis, are not specific for any type or condition of erythropoiesis.

SUMMARY

The incidences of plurinuclearity and pluripolar mitoses among erythroblasts in sternal marrows of eight normal individuals have been determined to be 1.0 to 5.1, and less than 1.0 per 1,000 uninuclear erythroblasts, respectively.

The incidences of such changes in eight pathologic cases have been shown.

The reversible nature of the phenomena of plurinuclearity and pluripolarity among erythroblasts of normoblastic, dysplastic, and megaloblastic types has been demonstrated.

The significance of the findings has been discussed.

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